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Cancer Survivorship

Edited by Dil Afroze

CANCER SURVIVORSHIP

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Notice

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Meet the editor

Dil Afroze, PhD, is Professor and Head of the Advanced Centre for Human Genetics. She received her PhD from Jamia Millia Islamia and has a master's in Biomedical Sciences. She is also Professor of Immunology and Molecular Medicine and has served the department for the last 20 years. Dr. Afroze has a long-standing research career that predates her appointment in immunology. She has worked as an immunologist at the Sher-i-Kashmir Institute of Medical Sciences, Srinagar, J&K, India. Dr. Afroze also serves as a member of the Indian Immunology Society and is currently its vice-president.

Contents

Preface

The increasing numbers reflect improvement all over the cancer continuum, which includes early detection, supportive care, and therapeutic approaches. Despite the considerable momentum that the topic of cancer survivorship has gained in the last few decades, an enormous amount of work remains to be done to achieve success throughout the cancer trajectory, which is detailed in the enclosed articles. This edition on cancer survivorship is the first issue in the IntechOpen Series, with its broad spectrum of articles devoted to cancer survivorship, and will provide an important update to survivors, clinicians, and researchers. When we initiated setting up the special edition, there were no previous examples to pursue and we were privileged that our editor-in-chief, Professor Dil Afroze, provided us the opportunity to complete the book. Moreover, this issue will continue to provide the field with the attention that it so importantly deserves. Because cancer is becoming a chronic condition itself and the prevalence of chronic conditions in the world population is at an all-time high, this book aims to provide state-of-the-art articles on topics important to the management of cancer clinically and answer some of the questions related to the interface of cancer and comorbidity that oncology practitioners face every day. We were directed by a number of principles, including that the articles should be evidence based, of the highest quality, and understandable by the practicing clinicians and oncologists. We also desired to guarantee that past advances at the molecular–clinical interface were covered, and that authors were also provided an opportunity to speculate on future opportunities. The resulting edition is a marvelous compendium of eight articles that cover a wide range of topics, including breast cancer: management and survivorship, rectal cancer intersphincteric resection, head and neck cancer diagnosis and radiotherapy, synthetic peptides as antitumor agents, and recent advances in thyroid cancer. It has been a wonderful opportunity to co-edit this special edition. We are greatly appreciative of the work of all the contributors to the book who brought with them tremendous diversity of perspectives and fields truly reflective of the complexity of the topic and who, through coming together in this project, serve as the nidus of the multidisciplinary collaboration in this field. Finally, we must acknowledge the thousands of cancer patients who have participated in the studies that have provided the information that has advanced the field so greatly in recent years.

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Chapter 1

Overview of Important "Organs at Risk" (OAR) in Modern Radiotherapy for Head and Neck Cancer (HNC)

Trinanjan Basu and Nithin Bhaskar

Additional information is available at the end of the chapter

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Abstract

With the advent of highly conformal and adaptive radiotherapy techniques, the significance of accurate delineation of organs at risk (OARs) is becoming more and more important. Techniques such as Intensity modulated radiotherapy (IMRT) and intensity/volumetric modulated arc therapy (VMAT) has allowed for improved dose conformation within the target. It has also allowed for steep dose gradients around the target for better normal tissue sparing. The accurate contouring and delineation of the OARs are thus warranted as variation in delineation has been systematically reported in studies. All these facts have led to the development of contouring guidelines for OARs in various sites. Head and neck cancers (HNC) are a perfect example where outcome and quality of life (QOL) balance remains a therapeutic challenge. There are several OARs and thus the accurate delineation following a standard guideline becomes more important. This chapter looks into the published guidelines for the delineation of such structures.

Keywords: head and neck cancer, IMRT, VMAT, QOL, organs at risk

1. Introduction

The variation in the contouring and delineation of the different OAR's has been systematically reported in studies [1]. We will be discussing the standard available guidelines and a simple practical way to delineate OAR's in modulated radiotherapy for HNC. The OAR's have been divided into five subgroups viz. optic structures, salivation related structures, structures related to swallowing, brachial plexus and intra-cranial structures.

2. Optic structures

2.1. Eye ball

The entire eye ball is to be contoured as a single structure. The entire retina is to be included.

For contouring of substructures of the eye the European particle therapy network (EPTN) has put forward a consensus based atlas based on CT and MRI [2].

2.1.1. Cornea

The cornea is located anterior to the vitreous humor, iris, lens and ciliary body [2]. It can be delineated in MRI or CT and is contoured with a 2-3 mm brush.

RT can injure the cornea by damaging the deeper layers of stroma, but in most cases the acute toxicity is as a result of loss of tear film [3].

Dose recommendation: < 40 Gy. Edema of the corneal stoma appears at a dose of 40-50Gy, but is usually transient. With doses of 60 Gy the chance of corneal ulceration is increased to 17–20% which increases further if chemotherapy is added.

2.1.2. Retina

It is the innermost layer of the globe and is about 0.25 mm in thickness and is not usually visualized in a standard MRI [2]. Contoured using a 3 mm brush, the retina covers the posterior 5/6th of the globe. The optic nerve is not contoured along with it [4].

Dose recommendations: Dmax—45Gy. Acute retinal toxicity is not reported. Being a part of the central nervous system, the retina behaves as a late reacting tissue [3]. Usually there is a latent period of 6 months to 3 years before the onset of clinically significant retinopathy. The mean latent period is 19 months [5].

2.1.3. Lens

Biconvex structure in the aqueous humor, it is clearly visible in CT [2]. The structure is about 10 mm in diameter seen in the coronal plane.

Dose recommendations: Dmax—5 to 10 Gy. Acute lens toxicity is not reported. A single dose of 2 Gy can cause cataract, but is usually visually insignificant. [3] The time of onset is dose related. For doses in the range of 2.5–6.5Gy, the latency is 8 yrs. with the possibility of 33% progressive cataract, whereas doses of 6.5–11.5Gy, the latency reduces to 4 years with the 66% risk of progressive cataract.

2.2. Optic nerve

While moving craniocaudally, optic nerve is seen below the superior rectus. The nerve is 2-5 mm thick and is delineated from the posterior margin of retina and continued along its course posteriorly till it merges with the optic chiasm after passing through the superior orbital fissure.

Dose recommendations: Dmax <54 Gy. The incidence of radiation induced optic neuropathy (RION) is unusual for doses less than 55 Gy. At 55–60 Gy the risk becomes 3–7% and for doses >60 Gy the risk is quite significant at 7–20% [6].

2.3. Optic chiasm

A small structure is usually confined to 2 or 3 slices in the superior-inferior direction. Better demarcated in MRI, the chiasm is situated about 1 cm superior to the pituitary gland. Laterally it is bounded by the carotid arteries. It is better visible in MRI with a high signal on T1. A good landmark to look for is the pituitary stalk. It lies just posterior to the chiasm and appears hyperintense even on plain CT [7]. On average it measures 8 x 14 mm (APxTrans) and is about 2–5 mm thickness in the super-inferior dimension [7].

It should be kept in mind that, the chiasm should be contoured in continuity with the optic nerves.

Dose recommendations: D max <54 Gy [6].

2.4. Lacrimal gland

Freedman et al. [8] has given a step-by-step instruction to contour the lacrimal gland. The contour starts by identifying the mid portion of the gland and thereafter tracking it superiorly and inferiorly.

Superior extend corresponds to the super-lateral corner of orbit, just below the orbital rim. Inferiorly it does not extend below the level of insertion of lateral rectus.

The gland is better delineated in brain (120/40) or soft tissue (350/50) window.

Dose recommendations: Dmean <30 Gy. Doses above 40 Gy have shown to steeply increase the incidence of dry eye while doses above 57–60Gy can cause permanent loss of tearing [9].

The details are given in **Figures 1–3**.

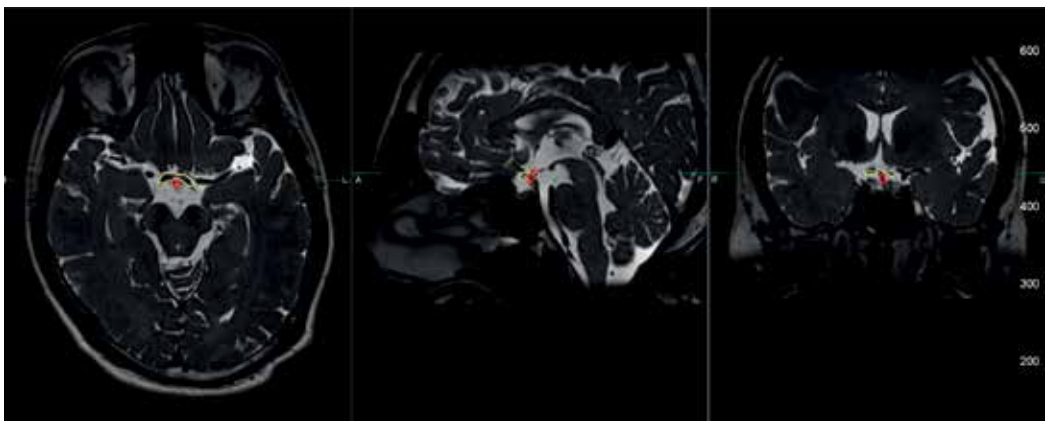

Figure 1. Optic apparatus.

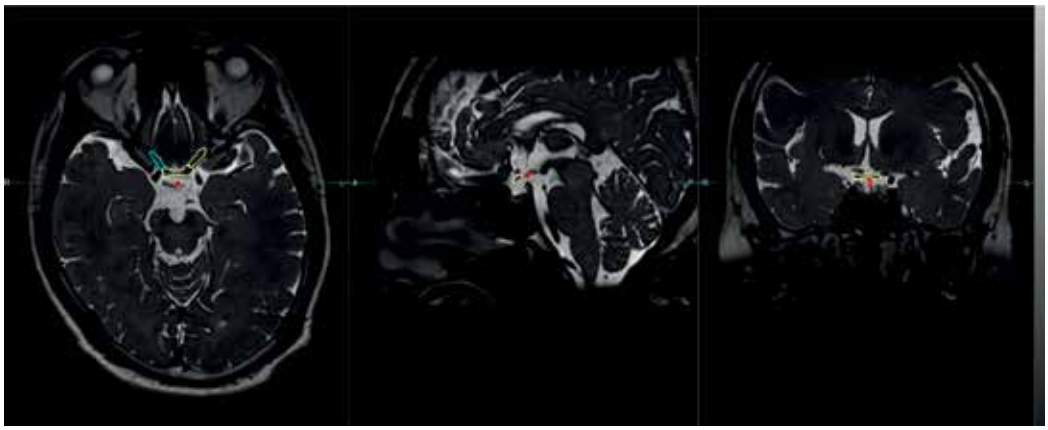

Figure 2. Optic chiasm.

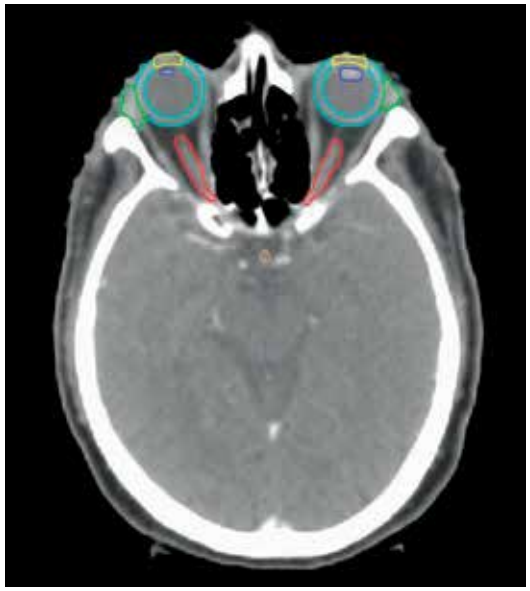

Figure 3. Eye balls and associated structures.

3. Salivation related structures

3.1. Parotids

Parotids are contoured based on the guidelines given by Water et al. [10] (**Table 1**). It is to be noted that in 20% of the cases, the parotid gland extends anteriorly over the surface of the masseter muscle following the parotid duct and in the anterior direction the deep lobe of the parotid gland may extend alongside the medial border of the mandible gland may extend

OAR	Anatomic boundaries					
	Cranial	Caudal	Anterior	Posterior	Lateral	Medial
Parotid gland	External auditory canal, mastoid process	Posterior part of submandibular space	Masseter muscle, posterior mandible, medial and lateral pterygoid muscle	Anterior belly of SCM muscle,	Subcutaneous place, platysma muscle	Posterior belly of digastric muscle, styloid, parapharyngeal space
Submandibular gland	Medial pterygoid muscle, mylohyoid muscle	Subcutaneous fat	Mylohyoid muscle, lateral surface, hyoglossus muscle	Parapharyngeal space, SCM	Mandible (medial border), platysma, medial surface of medial pterygoid	Superior and middle PCM, anterior belly of digastric muscle, mylohyoid muscle (lateral surface), hyoglossus muscle
Sublingual gland	Mucus membrane covering FOM	mylohyoid muscle, geniohyoid muscle	Mandible, mylohyoid muscle	Hyoglossus muscle	Medial surface of mandibular bone, mylohyoid muscle	Genioglossus muscle
Soft palate	Hard palate, nasopharynx (air space)	BOT, Tonsils, oropharynx (air space)	HP, BOT/tongue, oral cavity (airspace)	Pharynx (mucosal surface/airspace), superior PCM	Pterygoid process, medial pterygoid plate, superior PCM, medial pterygoid m., PPS, palatine tonsil, pharyngeal lumen	
Upper lip glands	Hard palate, nasal spine	lower end of upper lip	Orbicularis oris muscle, subcutaneous tissue/fat	Teeth, maxillary bone, HP, tongue	Depressor anguli oris muscle, buccinator muscle, levator anguli oris muscle/ risorius muscle	
Lower lip glands	Upper edge of lower lip	lower edge of teeth sockets/ mandibular body	Orbicularis oris muscle, subcutaneous tissue/fat	Teeth, mandible, tongue/air	Depressor anguli oris muscle, buccinator muscle,	
Buccal mucosa glands	Line between maxillary process and alveolar process of maxilla	Alveolar process of mandible	Orbicularis oris muscle	Posterior edge of mandibular body and maxilla	Buccinator muscle, subcutaneous fat	Mandible, teeth, tongue

SCM—sternocleidomastoid, PCM—pharyngeal constrictor muscle, BOT—base of tongue, HP—hard palate, FOM—floor of mouth.

Table 1. Salivation related structures.

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alongside the medial border of the mandible. The external carotid artery, the retromandibular vein and the extracranial facial nerve are enclosed in the parotid gland.

The external carotid artery (ECA), retromandibular vein and the extra-cranial part of facial nerve are enclosed in the gland. If contrast agents are used, the vessels can be clearly demarcated and can be avoided from the gland contour. But as contrast administration is not routinely practiced, and to make the contouring practice uniform, it is recommended to include the vessels within the gland contour.

Dose recommendations: severe xerostomia or salivary output <25% of baseline can be avoided if at least one parotid is restricted to a mean dose <20 Gy or both parotids restricted to <25 Gy [11].

3.2. Submandibular gland

Situated in the floor of the mouth, it is a predominantly serous gland having a large superficial lobe and a small deep process separated by the fibers of mylohyoid. In most cases the gland is hypo dense on CT and can be easily demarcated.

Dose recommendations: if deemed oncologically safe, the mean dose to the submandibular gland, restricted to 35Gy may reduce xerostomia symptoms [11].

3.3. Sublingual gland

Sublingual gland is the smallest of the three major salivary glands. It is a predominantly mucus gland, situated in the anterior part of oral cavity in the sublingual space.

3.4. Extended oral cavity

The extended oral cavity is contoured partly based on the work by Hoebers et al. [12] excluding the lips and buccal mucosa [4]. It includes the space posterior to the arch of mandible and maxilla. Posteriorly it is limited by the uvula, soft palate and the base of tongue.

3.4.1. Soft palate

Soft palate contains numerous minor salivary glands. It is better seen in the sagittal sections by a thin air line separating it from the tongue inferiorly. As the salivary glands are distributed along the length of the soft palate, the entire soft palate is contoured including the uvula.

Dose recommendations: oral cavity dose should be kept as low as possible. Seeking for V45 < 40% and V50 < 20% limits mucositis and improves QoL [12].

3.5. Other minor salivary glands

These glands are distributed along the inner aspects of lips and buccal mucosa between the mucous membrane and the muscle layer. Maximum depth from the mucosal surface is about 4 mm with lower lip glands deeper than the upper ones.

3.5.1. Lower lip glands

The upper and posterior limit of the lip is better identified in sagittal sections. The lower limit corresponds to the caudal limit of teeth sockets or the cranial mandibular body (in case of edentulate mandible) [10].

3.5.2. Upper lip glands

As of the lower lip, the lower and posterior extend is more easily made out in the sagittal plane. Cranially it extends till the nasal spine [10].

Dose recommendations: seek for a lip dose less than the oral cavity dose. A mean dose of 30Gy and 50 Gy for oral cavity and non-oral cavity cancers respectively would be preferable.

Orbicularis oris muscle can be used to delineate the glands anteriorly.

3.5.3. Glands of buccal mucosa

The glands of buccal mucosa are difficult to distinguish. The cranial, caudal and medial borders are better visualized in the coronal plane.

The abovementioned have been summarized in **Table 1**: salivation-related structures.

4. Swallowing related structures

4.1. Pharyngeal constrictor muscles

Pharyngeal wall has two layers of muscle (**Table 2**). The outer circular layer which are the pharyngeal constrictor muscles (PCM) and inner longitudinal muscles which are levators (stylopharyngeus and palatopharyngeus). PCM has three parts—superior, middle and inferior constrictor. The caudal ends of the levators blend with the PCM. These muscles are usually hard to differentiate from PCM and are not contoured differently.

4.1.1. Superior PCM

They originate from sphenoid bone from its pterygoid hamulus and insert to the median raphe. Different authors have put forward different levels in regards to its cranial border. Generally, cranial border of the superior PCM is taken as the caudal tip of the pterygoid plate, i.e., the pterygoid hamulus. The lowest fibers of the superior PCM are separated by the muddle PCM by stylopharyngeus and glossopharyngeal nerve. These fibers also overlap onto the upper fibers of middle PCM. As these changes are hardly made out in CT, most authors define the lowest limit of the muscle as the cranial border of hyoid bone. But this can lead to missing of half of middle PCM. Thus, the cranial border can be considered at the lower border of second cervical vertebra.

Organ at risk	Anatomic boundaries					
	Cranial	Caudal	Anterior	Posterior	Lateral	Medial
Superior PCM	Inferior tip of pterygoid hamulus	Inferior edge of C2	Pterygoid hamulus, BOT, pharyngeal lumen	Prevertebral muscles	Medial pterygoid muscles	Pharyngeal lumen
Middle PCM	Superior edge of C3	Inferior edge of hyoid	BOT, hyoid	Prevertebral muscles	Hyoid—greater horn. Thyroid cartilage—superior horn	Pharyngeal lumen
Inferior PCM	First slice inferior to inferior edge of hyoid	Inferior edge of arytenoid cartilage	Soft tissue of supraglottis/glottis	Prevertebral muscles	Superior horn of thyroid cartilage	
Cricopharyngeal muscle	First slice inferior to arytenoid cartilage	Inferior edge of cricoid	Posterior edge of cricoid	Prevertebral muscles	Thyroid gland/cartilage, fatty tissue	
EIM	First slice inferior to cricoid	1 cm inferior to the upper extend	Tracheal lumen	Prevertebral muscles	Thyroid gland, fatty tissue	
Cervical esophagus	1 cm inferior to the cricoid	Sternal notch				
BOT	Inferior edge of C1	Superior edge of hyoid	Posterior 1/3 from mandibular bone to pharyngeal lumen	Pharyngeal lumen	Width of the lumen of pharynx	
Supraglottic larynx	Tip of epiglottis	First slice superior to the arytenoid cartilage	Hyoid, thyroid cartilage, preepiglottic space	Pharyngeal lumen, inferior PCM	Thyroid cartilage	Pharyngeal lumen (lumen to be excluded)
Glottic larynx	Superior edge of arytenoid cartilage	Inferior edge of cricoid cartilage (only the soft tissue element)	Thyroid cartilage	Inferior PCM, pharangeal lumen/cricoid	Thyroid cartilage	Pharyngeal lumen (lumen to be excluded)

Table 2. Swallowing related structures.

4.1.2. Middle PCM

The fibers originate from the greater and lesser horns of hyoid bone and insert along the median raphe. The upper fibers overlap, therefore these boundaries are arbitrary. The upper border is taken as the lower border of superior PCM. Lower border corresponds to the lower border of hyoid bone.

4.1.3. Inferior PCM

The thickest of the three constrictors, the inferior PCM has two parts—the thyropharengeal part which originate from the oblique line of thyroid cartilage and the cricopharyngeal part which originate from the lateral part of thyroid cartilage. Most authors delineate these two structures separately. The thyropharyngeus is referred to as inferior PCM and the latter is referred to as cricopharyngeal muscle. As functionally these muscles are different, they are contoured differently.

The inferior PCM is starts cranially from the caudal end of middle PCM, usually one slice below the caudal end of hyoid. The caudal border corresponds to the upper border of cricoid, just below the level of arytenoid.

Anteriorly, the inferior PCM attaches to the posterior edge of thyroid cartilage, which can be recognized easily on CT, while the posterior border is defined by the prevertebral muscles.

Dose recommendations: Dmean < 50 Gy.

4.1.4. Cricopharyngeus

Delineation starts cranially one slice below the level of arytenoids which also corresponds to the lower limit of inferior PCM. The contour continues till the lower end of cricoid cartilage.

4.2. Esophageal inlet muscles (EIM)

Levendag et al. [10] recommends contouring the proximal 1 cm of esophagus as a separate structure. The cranial border starts from the caudal end of cricopharyngeus.

4.3. Cervical esophagus (CE)

The contouring of CE varies from authors [13, 14]. For the purpose of consistency the upper border of CE starts 1 cm below the esophageal inlet muscles and end at the level of sternal notch.

4.4. Base of tongue (BOT)

The delineation of BOT has been provided by three authors. All of them are consistent with the cranial extend defined as just below the soft palate. As this boundary is difficult to identify in CT, the lower end of anterior tubercle of C1 vertebra (which corresponds to the same level) may be taken for the demarcation. The three authors vary in the definition of the lower

extend. The lower limit of hyoid [13], the vallecular and the first slice of epiglottis [14] are mentioned, but for consistency, we follow the upper end of body of hyoid as the lower caudal limit of BOT.

4.5. Contouring of larynx structures

Freedman et al. [15] has provided a 3 step method to delineate larynx. Step 1 and 3 identifies the cranial and caudal limit of larynx. The contouring starts form the slice just below the caudal edge of hyoid and ends where the cricoid cartilage is seen as a complete ring. Step 2 mentions the circumference limits of the larynx. The anterior border corresponds to the inner surface of the thyroid cartilage. The posterior border in the upper part corresponds to the lateral surfaces of the aryepiglottic folds and the posterior surface of the mucosa covering the arytenoids. In the lower part it corresponds to the posterior surface of the cricoid cartilage. The pyriform sinus is not to be included in the contour.

The above guidelines contour the larynx as a single structure. As the larynx consists of subglottic, glottic and the supraglottic area, several authors have delineated these sub-sites separately. The supraglottis includes the epiglottis, the aryepiglottic folds, the arytenoids, and the false vocal cords. The glottis is composed of the true vocal cords and the subglottis extends from lower end of glottis to lower edge of cricoid.

The delineation of supraglottis and glottis is based on the function of the two subsites. While the supraglottis includes the muscles responsible for the closure of larynx, the glottis part is responsible for the movement of the vocal cords.

4.5.1. Supraglottic larynx

The contour includes the supraglottic adductors (oblique arytenoids and aryepiglottic muscles) and epiglottis. The cranial border is the tip of the epiglottis and the contour continues inferiorly till the upper edge of arytenoid cartilage.

4.5.2. Glottis

The contour starts from the upper end of arytenoid cartilage and ends caudally at the lower edge of the cricoid. Only the soft tissue is contoured (except for the arytenoids). The cricoid and the thyroid cartilage should be excluded.

Dose recommendations: To minimize laryngeal edema, the volume of larynx receiving 50 Gy and mean dose should be kept as low as possible, ideally ≤20% and 40 Gy respectively [16].

The above has been summarized in **Table 2**: Swallowing related structures.

5. Brachial plexus

Guidelines for contouring brachial plexus has been put forward by Hall et al. They have put forward step-by-step technique. The contour starts form the neural foramina of C5-T1.

It extends from outer edge of spinal canal to the space between anterior and middle scalene. Where no spinal foramina was present, only the space between anterior and middle scalene is contoured. The middle scalene will end in the region of the subclavian neurovascular bundle. In the lower part, the brachial plexus is contoured in the posterior aspect of neurovascular bundle in the inferior and lateral aspect one to two slices below the clavicle.

Dose recommendations: Dmax <60Gy. Emami et al. [17] has suggested TD 5/5 for the entire brachial plexus to be 60Gy. More recent studies with longer follow-up (upto 20 years) have shown that the risk of plexopathy keeps rising even after 5 yrs. and may not be apparent until 20 years after radiation [18].

6. Intra cranial structures

6.1. Ear structures

Ear structures (both the middle ear and inner ear) should be contoured using the bone window.

6.1.1. Middle ear

The eustachian tube (ET), tympanic cavity and the mastoid air cells (M) may be contoured separately based on the CT/MRI anatomy.

Dose recommendations: ET D_{30} < 52Gy; M $D_{0.05cc}$ < 41 Gy. Based on the study by Yao et al. [19], dose to 30% of ET and 0.5 cc of mastoid volume were the main predictors of severe ear disorders. Doses above these are associated with increase in grade 2 ear disorders post RT.

6.1.2. Cochlea

It is a small spiral structure of about 0.6cm^3 volume located in the petrous part of temporal bone. The small bony cavity can be visualized better with a setting of 120/1500 on CT. The structures of inner ear are visualized more in T2 weighted MRI images. The semicircular canals should not be contoured.

Dose recommendations: Dmean <45 Gy. In children it is advisable to keep it below 35 Gy. [20]

6.1.3. Vestibular and semicircular canal

Arranged in 3 planes, the canals are contoured in bone window (120/1500). They are located lateral and superior to the cochlea.

6.2. Brain stem

Brain stem comprises of midbrain, pons and medulla. The cranial extend starts from the level of inferior section of lateral ventricle. The organ is better visualized better in MRI. The contour extends till the level of the tip of dense of C2 vertebra or foramen magnum.

6.2.1. Midbrain

It starts from the nigral substance of the cerebral peduncle and ends upper border of pons.

6.2.2. Pons

Better visualized as an oval structure in sagittal sections, it is easily delineated.

6.2.3. Medulla

Medulla starts from the lower end of pons to the lever of tip of dense of axis.

Dose recommendations: Dmax—54 Gy. The entire brain stem can be treated to a dose of 54 Gy with little risk of serious side effects. [21]. Mean time of onset of symptom is 17 months (range 4.5–19 months). Smaller volumes (1-10 cc) may be irradiated to 59Gy at fractionations ≤2Gy [22].

6.3. Pituitary gland

A small gland, it is difficult to visualize in CT, but sella turcica can be used as a surrogate marked and the inner boundary of the same can be contoured for its delineation. The gland lies immediately below the brain and is connected to the hypothalamus by its stalk.

CT density of pituitary gland is similar to brain. Upon contrast administration, the gland may become more hyperintense than brain due to the rich vascular supply.

Dose recommendations: DMax 45Gy (for pan hypopituitarism, lower for Growth hormone (GH) deficiency). The anterior pituitary has 5 different types of cells, each with different radiosensitivity. Most sensitive is the GH axis followed by the gonadotropin, ACTH and TSH axis. GH deficiency has been noted in relatively lower doses, and has been reported for TBI for doses as low as 10 Gy [23], but the incidence increases substantially after 30 Gy where the incidence can be as high as 50–100%.

6.4. Temporal lobe

Contouring of temporal lobe should include the hippocampus, parahippocampal gyrus and the uncus. The basal ganglia and insula are excluded from the contour. Cranially it starts for the superior end of sylvian fissure and ends inferiorly at the base of middle cranial fossa. Medial boundary is marked by cavernous sinus, sphenoid sinus and the sylvian fissure and laterally by the temporal bone.

7. Discussion

The modern radiotherapy in HNC have revolutionized treatment outcome especially in terms of acute and late toxicity. It thus brings about a clear change in treatment outcome. One important aspect in preserving the QOL is the OAR's. We, as radiation oncologist are much

aware about the importance of accurate delineation of these structures. Only an accurate delineation can lead to effective sparing and thus a desirable outcome in terms of QOL. There were several isolated guidelines available. In this chapter we tried to summarize all the available guidelines. For certain organs like temporal lobe, multiple guidelines are available in the literature. We have tried to incorporate them together to put forward a single uniform consensus. Having said that the delineation and the attempted dose constraints should also be evaluated based on the target volume and tumor control. In case of parallel structures, the target volume coverage should be made priority and the risks and side effects of the same should be communicated to the patients. In such cases the volume of OARs outside the planning target volume (PTV) may be delineated separately and similar dose constrains may be aimed for. It should also be kept in mind that even with the most sophisticated of technologies, not all of the dose constraints might not be achieved due the basic physics of the photon beam. In such situations a trade-off should be agreed upon. However, such liberties, should not be attempted with serial structures like spinal cord and brain stem. Organs such as these should always be given hard constraints. If the PTV is overlapping such structures, under dose the area is accepted.

8. Conclusion

The chapter summarizes basic day to day information for a radiation oncologist to delineate OAR's in HNC radiotherapy. There are several updates and the readers are encouraged to go through them at a regular interval. We will be publishing a detail clinical end point based acute and late toxicities of these OAR's at a later date.

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Chapter 2

Breast Cancer: Management and Survivorship

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Additional information is available at the end of the chapter

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Abstract

Breast cancer is one of the most common in female population worldwide and comprises about 22.9% of all cancers. Despite the prognosis and survival rates of breast cancer patients and survivors are comparatively better than other cancers, but their net outcome can be revealed by other factors like tumor grade, secondary effects of chemotherapy like insomnia and health behaviors, this distressing may decrease patient's life expectancy. In the backdrop of this, the need of the hour for the breast cancer survivors is to assess multifactoral nonpharmacological interventions and the management that includes physical exercise, psychological and complementary medicine, which could be cost effective, widely accessible and more promising for breast cancer patients and survivors apart from pharmacological interventions.

Keywords: breast cancer, management, survivorship, prognosis, chemotherapy

1. Introduction

Breast cancer is the most common noncutaneous form of cancer and principal cause of cancer related deaths among females worldwide [1, 2]. Globally with an estimation of more than 1.38 million new cases (around 23%), the breast cancer ranks second (10.9%) among all cancers [1, 3]. In 2017, approximately 252,710 women and 2470 men cases were diagnosed with breast cancer. Approximately around 40,610 women and 460 men were expected to die from breast cancer in 2017 [4]. The survival rates and prognosis for breast cancer are mostly dependent on the type of cancer, stages, treatment, and the ethnicity and location of the patient. High survival rates have been observed in breast cancer cases of western world as compared to developing countries where survival rates are less. More than 3.5 million women with a breast cancer are still alive in the United States and are either having cancer or undergoing

treatment [4, 5]. The incidence rates of breast cancer vary from 19.3 per 100,000 women in Africa to 89.7/100,000 women in Europe [6]. The highest incidence (≥80/100,000) in developed parts of the world (excluding Japan) and the lowest incidence (≤40/100,000) in most of the developing part the world [7, 9]. There are a number of factors responsible for causing breast cancer. Dietary factors, such as high-fat diet, alcohol intake, smoking, obesity, higher levels of cholesterol, and iodine deficiency have high risk of cancer occurrence. Due to the highest incidence of breast cancer in USA, Australia, New Zealand and UK, makes them top priority countries for breast cancer awareness [8, 9]. It is well documented that breast cancer is strongly related to age; only 5% of all breast cancers occur in women are less than 40 years of age and more than 80% of all female breast cancers arise among women aged 50 or more years [10]. In general the age of a woman is directly correlated with higher risk of developing breast cancer. The majority of breast cancers are not hereditary [11]. In about <5% of cases, breast-ovarian cancer syndrome occurs by inheritance including women having BRCA1 and BRCA2 mutation. The 90% of total genetics account to these mutations with a breast cancer risk of 60–80% affected cases. BRCA1 mutations predispose women to breast and ovarian cancers. BRCA1 in breast cancer has higher aneuploidy number than tumors, which do not have mutations in BRCA1. About 85% of breast cancers occur in women who have no family history of breast cancer [12].

From the recent past improved therapeutics have greatly increased the chance of survival. The median age at diagnosis of breast cancer is 61 years, whereas 43% are older than 65 years at diagnosis; therefore, cancer survivorship should be conduct in coordination with comorbidities related with aging [10, 12]. Around 61% will have confined disease, for which survival success are highest (5-year relative survival rates: 99% for localized-stage breast cancer vs. 25% for distant-stage breast cancer [13]. Long-term survival is common after breast cancer therapeutics, in the midst of a 5-year survival rate of approximately 90%; hence, addressing survivors' exclusive post-treatment requires decisive to providing quality health care [14].

The National Coalition for Cancer Survivorship and NIH defines individuals as survivors from the time of their diagnosis through the balance of their lives [15]. Owing to enhancement in cancer care, as well as earlier detection and improved therapeutics, the cancer survivors in the USA has been gradually mounting over the last 30 years (~12 million in 2007) and is likely to keep on to increase [16]. Cancer survivors also survive longer following cancer diagnosis (~5 million survive more than 10 years) and two-thirds are aged 65 years and above [17]. However, improved survival brings numerous challenges that varies from an high risk for cancer recurrence and the advanced development of second or other new primary cancers, to a host of chronic circumstances (e.g., osteoporosis, thromboembolic disease, and cognitive impairment, cardiovascular disease), and plethora of inconvenient physical and emotional symptoms [18]. Recent studies document that underlying pathophysiological changes in survivors' metabolic, immune, central nervous systems, and neuroendocrine including the sleep-wake cycle, contribute to these manifestations is emerging features in clinical practice is important for many reasons. First, breast cancer survivors make use of extra care during their

first 5 years after diagnosis than their age-matched controls, yet time and again report that their needs have not been met [19]. Second, poorly controlled features may lead to decreased adherence to adjuvant endocrine therapy [20]. Indeed, many reports document that only 50% adherence to completion of 5-year adjuvant endocrine therapeutic regimens, leading to poorer survival [21]. Third, although majority of the breast cancer survivors account comparable quality of life (QoL) with respect to age-matched controls and experience post-traumatic development after cancer, for a subset of survivors, physical and emotional features can upset their QoL [22].

Breast cancer survivors' success not only depends on their prognosis, but also on a host of other characteristics, including undesirable effects of cancer therapeutics, available to and accessibility of survivorship care, economic, social, medical care and cultural variables, QoL and health behaviors [23]. Considerable numbers of breast cancer survivors do not stick to approved health maintenance attract toward good health behaviors, such as avoid or low alcohol intake, cessation of smoking, maintaining good health and body weight and maintaining habit of physical activity, can drastically diminish survivors' risk of breast cancer recurrence [23]. However, benefits may be hampered by cancer-and therapeutics-associated morbidity, particularly, if the clinical features are causal to morbidity are not well regulated [24]. Therefore, of these improvement in cancer survivorship, many organizations for instance the US CDC have called for "public and medical health professionals to: (a) addressing the potential long-term and overdue effects of cancer and associated therapeutics on survivors' physical and psychosocial health, (b) offer survivors by means of harmonized care to deal with their several symptoms, and (c) foster the significance of healthy behaviors (e.g., cessation of smoking and physical exercise) to diminish the risk for new type or recurrent cancer and early detection to augment the chances of survival with new or recurrent cancer [2, 3]. QoL of enduring cancer survivors has been planned to be constituted of four principal domains of health: social, psychological, and spiritual physical. Given a number of inclusive overviews of the whole scenario of breast cancer survivorship QoL, we wanted to present an upgrade of the most recently published literature. Therefore, our focal point in this book chapter is to emphasize updated work in the field, of breast cancer survivorship and management, prioritizing from last few years of research, with intent of providing clinicians the realistic suggestion to enlighten their practices [25, 26].

2. Role of primary care providers (PCPs) in breast cancer survivors

Depending on number of survivor's, primary care physicians should have access to the evidence and be able to provide care and follow up. Breast cancer prevalence rates are alarming due to the growth and aging of the population [27]. Women are able to survive with breast cancer after treatment that follows up routinely to detect any recurrence and manage late and long-term outcomes of treatment [28]. Breast cancer survivors are

growing in number with increase in cost for their well-being, follow up and other medical care facilities [29]. The role of primary care providers is increasingly being taken up for follow-up care and same should be maintained as we have limited secondary care facilities [30]. There should be a systematic registry and data maintenance that follow up is being done and breast cancer survivors are effectively treated in primary care [3]. There is a short and long term side effect on health of breast cancer survivors including physical, emotional, sexual and social consequences such as pain, depression, fatigue, weakness [31]. The loss of confidence in leading a normal life is one of the increasing factors which is treated by primary care system and are involved more as compared to control patients [32]. It is important that PCPs should provide good and maximum care to cancer survivors and need of patient's should be satisfied. PCPs prefer to guide on disease recurrence and risk management involved in cancer treatment and survival of patient [32]. The evidence based recommendations are provided to PCPs for cancer survivors and are available for clinical practice guidelines [32]. Most of the recommendation is available on how recurrences occurs in a breast cancer patient and are concerned with diagnosis [3, 33]. Mammography has been recommended to breast cancer patients on follow-up in five guidelines and physical examination of breast in three of them [3, 33]. Ultrasound is recommended in one of the guideline while undergoing mammography [33]. All these guidelines advise genetic counseling for risk factors and patients are educated enough to know about signs and symptoms of recurrence or any other underlying cause and sign, how to resolve it and inform your medical advisor [3, 33].

3. Management strategies for breast cancer

Despite the prolonged survival and improved lifespan, there are a few manifestations that breast cancer survivors' physical utility might diminish more rapidly than that of their peers. These survivors might facilitate from an approach that is frequently applied in the aging population [34]. First, survivors encounters several, congregate and chronic features. Second, survivors display psychological, physical, and behavioral variations that may hamper their efficient status and lead to dependence and impairment, as revealed by higher fall risk, reduced physical exercise and distinguishes limitations in both basic and highly developed activities of daily living [3]. Thus, a subset of characteristic breast cancer survivors set to benefit from a management approach that is regularly applied in the elderly medicine situations, one that is: multidisciplinary (e.g., behavioral and physical factors); multifactorial (i.e., one intervention targets multiple symptoms simultaneously); augmented use of nonpharmacological treatment intervention options; and setting therapeutic goals (i.e., recognizing that complete resolution may not be achieved, but improvement is likely) [35] (**Table 1**). Various pharmacological and nonpharmacological interventions are used for the therapeutics of breast cancer as well as post therapeutics are shown in **Figure 1**. In a below section, we will demonstrate this concept by stressing basic measurement and management principles that apply evenly across numerous frequently

observed physical and emotional features in breast cancer survivors, such as adjustment disorder, cognitive dysfunction, or other vasomotor symptoms, psychosocial distress, and fatigue and insomnia.

Treatment type	Long-term shortcomings	Late shortcomings
Surgery	Loss of dermal sensation. Body shape change. Sexual disturbance. Impaired movement. Motion poor weakness	Lymphedema Neurological disorder
Radiation therapy to the breast including chest adjacent wall lymphatic nodes	Weakness Skin is sensitive to pain Impaired pain Pain Pneumonitis Poor dressing sense Breast atrophy Lymphedema Impaired pain or weakness of the upper limb	Skin color change Asymmetric breast Sexual disturbance Ataxia telangiectasia- Neural dysfunction lymphedema Breast volume not same in both affected and normal Difficulty in breath fibrosis Cardiovascular disease Second primary cancers arise of soft-tissue thorax, cervical, shoulder, lung cancer
Chemotherapy	Sexual disturbance fertility decreased Gain of body weight and mass, fat accumulation increases, neurological disorders, weight gain, oral, hair loss Weakness Ovarian dysfunction	Osteoporosis/osteopenia Cardiovascular risk factors at increased level increased risk of (cardiomyopathy, congestive heart failure) with chemotherapy Leukemic malignancies at increased risk with alkylating agents, anthracyclines, other topoisomerase II inhibitors
Hormonal therapy		
Tamoxifen	Hot flashes, menstrual cycle irregular, mood swings Increased triglycerides	Increased cardiovascular diseases, cervical cancer, blood clotting, bone malformation in premenopausal women
Aromatase inhibitors	Vaginal dryness, sexual dysfunction, muscle pain, neurological disorder, cholesterol increased	Osteoporosis, bone malformation, bone fracture
Targeted therapy		
Trastuzumab	Cardiac arrest, cardiac dysfunction	
Psychosocial long-term and late shortcomings	Mental depression, tension, headache, unpleasant behavior, social distress, physically un well, pain, end of life bad dreams, death fear, social difficulties, sexual problems, relations problems, financial shortcomings	

Table 1. Long term and short term short comings of breast cancer therapeutic interventions.

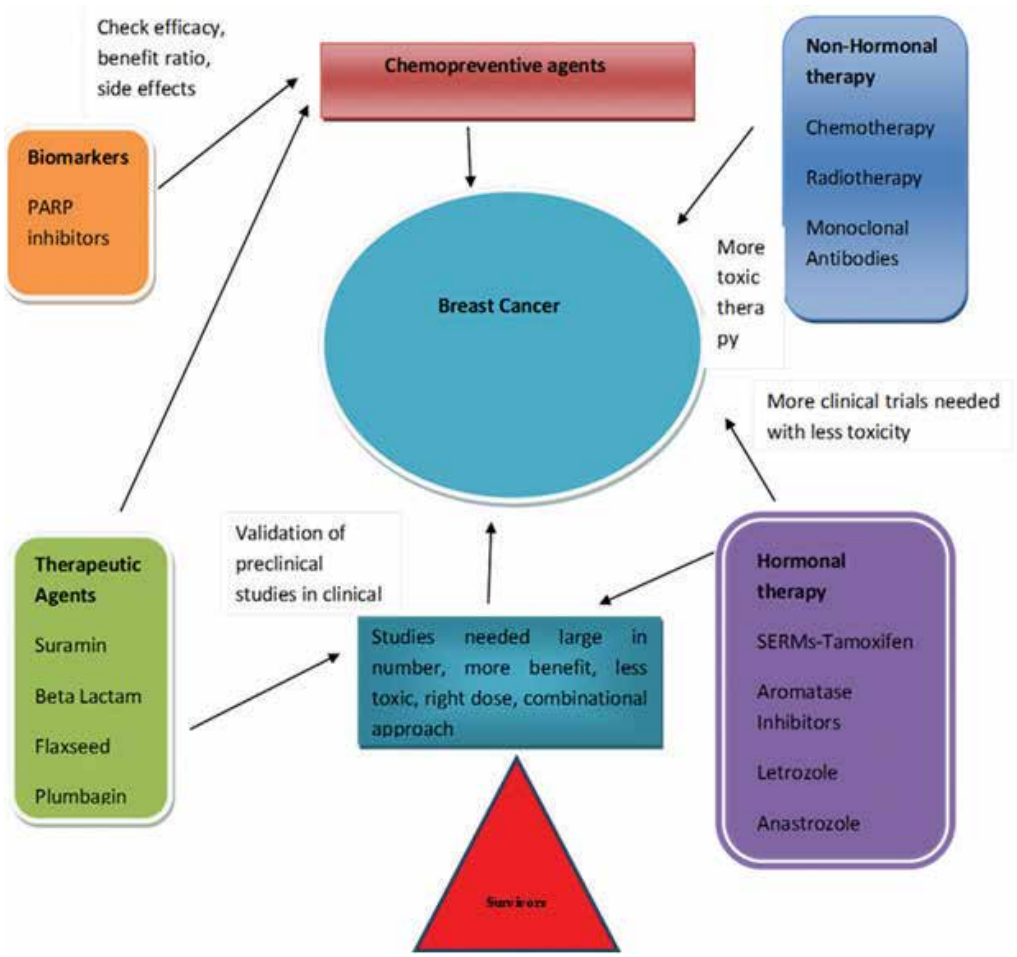

Figure 1. Different therapeutic targets in breast cancer treatment explain role of each new agent, its benefit to survivors.

4. Surgical treatment of breast cancer

Primary management of breast cancer is surgery. Most of the women diagnosed with early breast cancer underwent surgery. It may be wide local excision or mastectomy. Mastectomy is indicated when there is a presence of polycentric invasive cancer spots, inflammatory carcinoma, intra-ductal carcinomas, large primary tumors and patient preference [36]. Skin-sparing mastectomies followed by immediate reconstruction are now one of the most popular forms of reconstruction [36]. The status of the axillary lymph nodes is an important prognostic factor in early stages of breast cancer. During the operation, the lymph nodes in the axilla are also considered for removal. Axillary lymph node dissection remains the standard of care for patients with clinically palpable or positive histological confirmed lymph nodes [36]. Sentinel lymph node biopsy(SLNB) is an advanced technique and is very helpful in the management of axilla [37]. SLNB technique is based on the observations that tumor cells migrate from the primary cancer site to one of the nearby lymph nodes before moving to other distant lymph nodes [38]. In patients with a negative

lymph node biopsy, there is no requirement of axillary lymph node dissection (ALND) [39]. This in turn reduces the risk of lymphedema and other complications such as nerve injury and muscular problems, associated with the axillary lymph node dissection surgery [40].

5. Pharmacological interventions

5.1. Adjuvant chemotherapy for breast cancer management

Some patients with early breast cancer relapse with stage IV breast cancer even after surgery, reason may be the presence of micrometastatic disease present before the surgery [2]. For this reason in breast cancer patients adjuvant systemic therapy are used to halt the process of cancer recurrence [2]. It has been shown and proved by several trials that 6 months anthracycline therapy following surgical treatment of cancer results in about 40% reduction in mortality rates from breast cancer in women below age of 50 years and 20% reduction in between 56 and 69 age group [2, 41]. Further analysis showed that the addition of taxane based chemotherapy to anthracycline based regimen reduced mortality rate by further 15% [2, 41].

5.2. Adjuvant trastuzumab monoclonal antibody

At least 18–20% of breast cancer patients tumor cells overexpress HER-2 proteins [42]. In these patients trastuzumab (monoclonal antibody) is given as an adjuvant therapy [2, 42]. Full course of 52 treatments are given to woman with breast cancer [42]. Trastuzumab is well tolerated at therapeutic doses but may result in cardiac toxicity in patients initially given anthracycline regimen or in patients having baseline cardiac problem [43]. Left ventricular dysfunction and heart failure is reported in some patients by administration of trastuzumab [42]. All breast cancer patients should have scheduled cardiac monitoring before and during administration of therapy [2, 42].

5.3. Adjuvant endocrine therapy and breast cancer management

The majority of breast cancers are estrogen receptor (ER) positive [2, 42]. In those women with ER positive breast cancer, adjuvant endocrine therapy is routinely offered [2, 42]. The addition of endocrine therapy following either adjuvant chemotherapy or radiotherapy confers a further reduction in mortality rates [2, 42]. Tamoxifen is the mainstay of endocrine treatment in premenopausal woman [2, 42]. In post-menopausal woman aromatase inhibitors are the agents of choice [2, 42].

6. Non-pharmacological interventions

6.1. Psychophysiological symptoms

A thorough approach to evaluate the symptom management is to investigate in detail whether symptoms can be accredited to cancer and associated therapeutics and other procedures

including anesthesia or to additional contributing factors that are capable to be reversible [44]. These various factors might include anemia, vitamin deficiencies, organ dysfunction, metabolic, intracranial or endocrine abnormalities. The suitable investigations for various deficiencies can be assessed by methods which include imaging studies, blood work and/or neuropsychological testing. Additionally, the sensible and careful listening to survivors, education and multifactorial assistance from other disciplines (e.g., physical and behavioral medicine) associated to survivors may contribute to therapeutics significantly [45].

Management comprises of pharmacological therapeutic intervention (e.g., antidepressants or stimulants) in combination with a range of nonpharmacological interventions, for example psychological support, physical therapy, complementary medicine and nutritional counseling [46, 47]. The other choice to pharmacological therapeutic intervention for managing these side effects is psychological therapeutic interventions [48, 49]. Various behavioral interventions are linked with the diathesis stress model. This model explains how acute symptoms might develop into well-established or long lasting. However, many patients have predisposing factors to make them more susceptible to the event of insomnia. Such as, under the precipitating factor like stress, biologically older female patients sleep very less and make them prompt for low threshold for developing insomnia [50]. Owing to the cancer diagnosis and its therapeutics, undoubtedly accumulates more stress and advance in the development of acute insomnia. In an effort to mitigate their symptoms, patients may draw in behaviors that eventually perpetuate their insomnia. Cognitive Behavioral Therapy for Insomnia (CBT-I) proposed by Spiel man provide the foundation of this model [25]. For instance a patient may initiate to spend more time in bed or take more naps throughout the day in the hopes that this would lessen her tiredness or fatigue, when in fact it may intensify his/her insomnia by disturbing the sleep wake and circadian cycles [51]. A behavioral therapeutic intervention aims these perpetuating behaviors to decrease sleeplessness problems and assist patients develop a more regular and standard sleep schedule. A behavioral therapist would support the patient to circumvent compensatory methods, such as sleeping during the course of day or lounging in bed, or would initiate stimulus control methods, such as reserving bed for sleep and sex only to decrease the number of relations with bed that are not conducive for sleep (watching TV, worrying, staying awake). Some of these therapies influence multiple symptoms, which is the need of hour [1]. For example, increasing physical exercises not only develops functional status, strength and survival, but also facilitates improved QoL, emotional, cognitive and physical symptoms and on the whole cancer-associated mortality [52]. A sensible and well knowledgeable discussion can assist to delineate an individualized management approach that accounts for aforementioned therapeutics, patient preferences and insurance coverage. Pursuing nonpharmacological treatment management can be formidable, as it frequently entails more dynamic involvement of the survivor (e.g., time and expense). For example, cancer survivors might be challenged in their pursuit to commence, continue or enhance the height of physical exercise by logistical difficulties, attained physical constraints or a fear of injury. In these occasions, a proficient cancer exercise trainer or a physical therapist is an important resource. Cancer survivors may undergo discouraged when misery with long lasting symptoms, and even though most breast cancer survivors manage well, some survivors become significantly laden by symptoms, and in this case, a brief course of psychological therapy focused on empowerment, increased self-care and coping skills may prove helpful [53, 54].

6.2. Physical activity

Physical exercise has been proved to have potential to counter numerous cancer-associated therapeutic side effects. Scientific world has documented that proper physical exercise strongly related with improved outcomes. Various reports and meta-analysis revealed that proper and well managed physical exercise have many health benefits and could ameliorate anxiety, improved quality of life such as blood circulation, physical fitness, depression, improving body shape, remove depression, good impact on quality of life, cognitive impairment and decreases inflammation as well as diminishes toxicities stemming from deleterious cancer therapeutics [55]. Breast cancer survivors should be recommended to revisit to normal daily activities as quickly as possible after diagnosis and to continue engaging in regular physical activity [3, 55]. The guidelines recommended for cancer patients and cancer survivors reported in American College of Sports Medicine documents that one should perform moderate intensity aerobic activity includes gardening, walking and ballroom dancing for 150 min or strenuous aerobic exercise which includes jogging, race walking or hiking uphill for 75 min in a week and 20–30 min of moderate-intensity anaerobic muscle strengthening activity for at least twice a week [2, 3, 55]. Additional details about the amount of time required for each entire training session is less clear. More importantly cancer patient and survivor should inquire about the advice of oncologist and/or primary care clinician prior to initiate a new activity. However working with professional trainers who are skilled to perform such type of exercise for cancer patients and survivors, and acquire individualized exercise directions that account for different types of cancer, disease progression, comorbidities, physical limitations, age, and other appropriate factors [3]. Additionally preliminary reports document the fact that proper regular exercise from well trained person could confer a survival benefit and is related with or increase overall quality of life significantly across the cancer patients and cancer survivors. It is worth to mention that up to 70% of breast cancer survivors do not meet these ACSM public health recommendations and have not taken any opinion to discuss activity with respective oncology care team [56]. About 32% of cancer survivors meet the recommendations for physical activity. Observational evidence advocates greater amounts of physical exercise may be needed, even though the data is insufficient to make it an approval at this time; aerobic exercise of 3 h or more per week may possibly be needed to improve breast cancer survival [57]. However, reports revealed that cancer survivors of entire ages would like their oncologist to start discussion about activity and formulate suitable referrals timely in the therapeutic course [57]. Given the benefits of physical activity during and after the cancer therapeutics, schedule discussions about exercise between the oncologist team and cancer patients and survivors in combination with referrals to a trainer exercise physiologist could considerably advance cancer therapy adherence, side effect burden, recovery, quality of life, as well as disease-free and overall survival in breast cancer patients and survivors [3, 57].

6.3. Nutrition

The diet characterized by high content of vegetables, fruits, whole grains, and legumes (vs a typical Western diet) has been related with a low risk (range, 15–43%) in all-cause mortality. Only 18–34% of breast cancer survivors report eating five or more fruit and vegetables daily [58]. Data from the two large RCTs of diet therapy in breast cancer survivors and patients

suggest that the dietary change adequately result in weight loss and may possibly be required to favorably impact breast cancer recurrence and prognosis [3]. As per ACS nutrition and physical activity guidelines, alcohol consumption should be limited to no more than one drink per day for women, as in the general population [3]. Data are not consistent but advocate that breast cancer survivors who consume more than three to four drinks per week are at high risk for breast cancer recurrence. Various studies revealed that the carcinogenic ingredient of alcohol consumption augments the risk of developing numerous types of cancers irrespective of the type (i.e., wine, beer, etc.), and recommended limits [3]. About 7% of breast cancer survivors revealed the excessive drinking of alcohol. Based on these facts, survivors should be counseled to: achieve a dietary pattern that is high in vegetables, fruits, whole grains, and legumes; limit alcohol intake to no more than one drink per day; and follow the ACS guidelines on nutrition and physical activity for cancer survivors with intend on successful weight management [3, 59].

6.4. Musculoskeletal symptoms

Despite other deleterious effects, breast cancer survivors may account intricacies with the ipsilateral upper end after surgery, as well as decreased range of motion, rotator cuff injury, adhesive capsulitis ("frozen shoulder" with firmness and pain in the shoulder joint), and axillary web syndrome ("cording" in the skin of the inner arm with ambiance of pain and stiffness that emerge as a web or a corded rope) [3]. These malformations can lead to a decline capability to perform exercise of daily living and can effect employment [1, 3]. Other therapies like systemic therapy may also be associated with the progression of musculoskeletal symptoms in breast cancer survivors [1, 3]. Musculoskeletal symptoms are very common in the midst of breast cancer survivors, whether due to aromatase inhibitor therapy, menopause or chemotherapy, The incidence of musculoskeletal symptoms in breast cancer patients differs significantly: these include restricted shoulder range of motion (range, 1.5–50% of patients), musculoskeletal pain (range, 12–51% of patients), upper limb tiredness (range, 18–23% of patients), and immobility or numbness (range, 29–81% of patients). Above 50% of breast cancer survivors endure a musculoskeletal syndrome on initiation of an aromatase inhibitor, generally as either new or worsened diffuse arthralgias or myalgias, which most often develop within 2–3 months. Around 30% of breast cancer survivors entail to stop early adjuvant aromatase inhibitor therapy due to deleterious effects (~25% because of musculoskeletal symptoms) [1, 3, 60, 61]. In particular, up to 50% of postmenopausal women undergo therapeutics with aromatase inhibitor medications report arthralgias (joint pain) and myalgias (muscle pain) that are intense enough in 20% of women to lead to therapeutics discontinuation [1, 61]. These aromatase inhibitor-related musculoskeletal symptoms are frequently not receptive to nonsteroidal anti-inflammatory drugs or acetaminophen [1, 61]. Another option for treatment is alternative therapy that is replacement of one antiestrogen therapy with another. More than 40% of the patients who discontinue the drug may tolerate a distinct aromatase inhibitor or a discrete formulation of the aromatase inhibitor. The rest usually tolerate tamoxifen. Poor compliance/adherence to therapeutics has revealed the high risk of breast cancer recurrence, therefore serving breast cancer patients and survivors management and their symptoms and supporting drug compliance has utmost importance [1, 61]. Albeit a underlying relationship is possibly to be due to cancer therapeutics or

menopausal transition, new diagnostics, such as autoimmune or crystalline arthritis, should not be ignored. Treatment modalities are similar to those of osteoarthritis and may cover nonpharmacological methods such as physical therapy, local heat and/or acupuncture, in combination with pharmacological therapy, such as immediate short-term administration of NSAIDs, either systemically or topically [1, 3, 62, 63].

Besides arthralgia-type symptoms, breast cancer survivors may also progress fibrosis, rotator cuff pathology and distorted body habits, thereby leads to pain, limited range of motion and malfunctions in daily life, which are considered to be consequences of loco-regional treatment with surgery, with or without radiation therapy. Axillary web syndrome is the most extreme presentation of these symptoms. Physical and massage therapists are of great value as they are able to provide symptom relief by administering an intense regimen of massage, stretching and exercises that minimizes the need for surgical therapy. Physical therapy, including stretching and other exercises, has been reported to be efficient for managing postsurgical musculoskeletal symptoms. Recent reports from the hormones and physical exercise trial, a prospective cohort study, revealed that contribution in an intensive exercise regimen resulted in a 20% reduction in aromatase inhibitor-related pain. So far, only acupuncture and physical exercise have been reveled statistically significant improvement in aromatase inhibitor-related symptoms. Additionally psychological support may help patients cope with pain and can also be helpful [1, 3, 62, 63].

6.5. Infertility, sexual dysfunction and urinary complaints

Infertility as a consequence of chemotherapeutics is a potential enduring deleterious effects faced by younger breast cancer survivors (younger than age 45 years). More than half of breast cancer survivors, especially those on adjuvant endocrine therapy suffer from sexual dysfunction [1, 3, 64–68] and urinary symptoms [1, 69]. Sexual dysfunction may be caused due to vaginal atrophy, diminished libido or feeling of pain during intercourse [1, 3]. Changes in libido have multiple causes, including mood disorder, pain, fatigue, estrogen deprivation and issues in relationship [1, 3]. Sexual issues can be partially subsided by using non-hormonal approaches i.e. by using moisturizers, lubricants, vitamin E and low doses of topical estrogens [1, 3]. Pelvic floor muscles exercises, gynecologic, vaginal dilatational measures and psychological care are also very helpful [1, 3]. Treatment with aromatase inhibitors usually cause vaginal dryness, menopausal symptoms, and loss of sexual desire [1, 3]. Radiation therapy can often cause skin fibrosis, loss of sexual sensitivity of the skin, and, uncommonly, cardiac and respiratory damage, all of which negatively impacts on sexual desire and response [1, 3, 70]. It is important to counsel patients concerning possible sexual dysfunction remedies, including treatments for vaginal dryness [1, 3]. Nonhormonal, water-based lubricants and moisturizers remain the primary treatment [1, 3, 71]. Silicone based products or glycerin-based products also show very good results [3]. Hormonal therapies, such as a low-dose estrogen vaginal tablets or an estradiol vaginal ring, may be recommended for vaginal dryness because of urogenital atrophy, although better results are shown approximately after 6–12 week treatment [1, 3, 71, 72]. Urinary issues include overactive bladder with urgency with and without incontinence [1, 3]. Urinary complications can usually be reduced by pelvic floor muscle exercises, topical estrogen and, in some cases, by consulting a urogynecologist

[1, 3]. It is recommended that primary care clinicians should refer survivors of child bearing age who experience infertility to a specialist reproductive endocrinologist [1]. Infertility as a result of breast cancer treatment is a potential long-term side effect faced by younger breast cancer survivors. Infertility can have a profound impact on a survivor's physical and social character [1, 3]. Chemotherapy can be gonadotoxic, leading to reduced fertility or early menopause secondary to premature ovarian failure [1, 3, 73]. Many of the most frequently used chemotherapy agents in the treatment of breast cancer (e.g., alkylating agents and taxanes) are also those that most often lead to premature ovarian failure [1, 3, 74]. The incidence of chemotherapy-related amenorrhea has shown to be increased with age due the reason that the female ovarian reserve is nonrenewable and usually diminishes steadily with age [1, 3]. Primary care clinicians should involve the medical oncologist in any discussion related to the time for pregnancy after breast cancer treatment completion [1, 3]. Premenopausal women who desire pregnancy and are having difficulty conceiving for 6 months or more should be referred to a fertility specialist [1, 3]. Timely referral is crucial because of the rapid loss of ovarian reserve in these women [1, 3].

6.6. Management of bone health

It is estimated that osteoporosis affects one in every three postmenopausal women, with a 40% lifetime risk of fracture and approximately 21% risk of 1-year mortality after hip fracture [3, 75]. Breast cancer survivors are at very high risk of bone loss due to chemotoxic effects on the bones, due to treatment induced hypogonadism, due to supportive steroid therapies and due to vitamin D deficiency [3, 69]. Simple measures, such as weight-bearing exercises, avoiding of consumption of nicotine/alcohol and an adequate intake of calcium and vitamin D (600 mg/400 IU twice daily), are very essential for fracture risk reduction [1]. Breast cancer survivors should be monitored by dual-energy X-ray absorptiometry scans every 2 years [3]. Postmenopausal women treated with aromatase inhibitors are at increased risk of osteoporosis and should have periodic dual-energy X-ray absorptiometry scans [3]. All postmenopausal women or premenopausal women which are under GnRH agonist induced ovarian suppression therapy are at higher risk for developing osteoporosis and should be screened for postmenopausal osteoporosis diagnosis and treatment [1, 3]. In addition to lifestyle and nutritional interventions, pharmacologic options should be considered in patients, which are at high risk for bone loss [1, 3]. Bisphosphonates or denosumab can prevent bone loss and are good and established treatment options for osteoporosis [1, 3, 76, 77]. However, these drugs do have side effects and risks, so that the risk versus benefit must considered in a careful manner [3]. Estrogen receptor modulators raloxifene and tamoxifen could also be used because of their antiresorptive nature.

6.7. Obesity concerns after treatment

About 62% of breast cancer survivors are overweight, of which 30% are classified as obese [2, 3]. Various research studies have presented obesity as a risk factor for postoperative complications, risk of recurrence, development of diabetes, and other issues [78]. Conversely, weight

loss subsides symptoms and improves QoL. Weight gain during breast cancer treatment is a serious problem [78]. Breast cancer survivors should avoid inactivity and return as soon as possible to normal activities after surgery and therapy [79]. American Society of Clinical Oncology (ASCO) has issued a statement that guides oncologists to counsel their patients about the benefits a healthy weight [80]. Primary care clinicians should also counsel cancer survivors to achieve or maintain a healthy weight as well as counsel survivors, to limit consumption of high calorie foods, beverages, to increase physical activity and maintain healthy weight [1, 3]. Various research studies have shown that heavier survivors are more likely to die of cancer due to recurrence [3, 81]. Various suggestions and diet charts for getting back to healthy weight in breast cancer survivors have been recommended. E.g. eating at least 2 cups of vegetables and fruits, more usage of whole grain foods like brown rice, less intake of saturated fats, preference of chicken and fish over red meat and moderate aerobic exercise of 150 min per week has shown to reduce and subside the complications associated with breast cancer treatment [81].

6.8. Cardiovascular issues in breast cancer survivors

Breast cancer survivors are at increased risk of cardiovascular disease and the collective incidence rate is 33% [82] Breast cancer patients after treatment experience cardiotoxicity and it may also lead to cardiac issues like cardiomyopathy, thrombosis, pericardial disease and arrhythmias [82]. It is important to control and assess cardiovascular risk factors including weight, physical fitness, lipid profile and glucose tolerance [83]. A dietitian, psychologist and personal trainer may help facilitate these efforts [3]. It is recommended that primary care clinicians should monitor lipid levels, provide cardiovascular monitoring and should also educate breast cancer survivors on healthy lifestyle modifications, potential cardiac risk factors [3]. Radiation, chemotherapy, and endocrine therapy with aromatase inhibitors have been associated with an increased risk of cardiovascular disease in patients with breast cancer [84]. The risk of heart disease increases in postmenopausal women, as endogenous estrogens in younger women contribute to the low prevalence of cardiovascular disease in that population [3]. Various studies have shown that breast cancer patients who experience treatment-related early menopause are at higher risk for development of heart disease [3]. The chemotherapeutic agents epirubicin and doxorubicin are associated with an increased risk of cardiomyopathy [85]. Similarly, trastuzumab is associated with an increased risk of cardiac dysfunction, most notably when taken together with anthracycline [2, 3, 85]. Aromatase inhibitors are known to raise cholesterol levels and the increase the risk of diabetes in breast cancer patients after or during treatment [3]. Excessive weight gain may lead to hypertension and insulin resistance, which further elevate the risk of cardiovascular disease [3]. Primary care clinicians and physicians should monitor lipid levels as well as monitor cardiovascular changes in cancer patients after treatment [3]. It is very essential to educate breast cancer survivors about daily lifestyle modifications, like smoking cessation, diet, and exercise that may be helpful in reducing severity and risks of cardiotoxicity [3]. Patients should be advised to be aware of the potential cardiotoxicity risk and also advised to report symptoms such as shortness of breath or fatigue to their health care provider [3].

6.9. Lymphedema associated with breast cancer treatment

About 40% women breast survivors face a very higher risk of developing lymphedema [1, 3, 86]. Breast cancer related lymphedema results from obstruction of the lymphatic system due to removal of lymph nodes and tissue damages caused by radiotherapy [87]. Survivors personal habits like obesity and overweight can increase the chances of lymphedema [88]. Lymphedema has associated psychosocial problems that hamper day to day lives of breast cancer survivors [89]. Infections can trigger and worsen and the symptoms of lymphedema, so it is important to have good personal hygiene in order to reduce the risk of infection [3]. All patients in which lymphedema has developed should be referred to a physical or occupational therapist or to lymphedema specialists [3]. Management of lymphedema remains a major challenge for patients and health care professionals [3]. Routine check-ups for lymphedema management, physical therapy, use of bandages, special lotions and frequent infections creates a huge financial burden not only to breast cancer survivors but also to the health care system [86]. Breast cancer-related lymphedema can occur in shoulders, breast, and thoracic area [86]. Treatments include pharmacological therapy and adjuvant therapies [3, 86]. Drugs included in the pharmacological management of lymphedema are benzopyrones, flavonoids, diuretics, hyaluronidase and selenium [86]. Surgical treatment for lymphedema in breast cancer survivors included lympholymphatic anastomoses, debulking and liposuction [3, 86]. Chronic lymphedema in breast cancer survivors sometimes lead to formation of excess subcutaneous adipose tissue which can be removed by liposuction [86]. Liposuction increases the blood flow in skin capillaries without damaging already compromised lymph transport capacity in breast cancer survivors with lymphedema [1, 3, 86].

7. Strategies to improve breast cancer survivorship symptom care

7.1. Survivorship care plan

There has undoubtedly been a call from all over the globe to stress on the importance of largely on cancer survivorship and care. In recent past, first cancer survivorship symposium was held by ASCO, and highlights the crucial issues faced by cancer survivors and patients with numerous cancers following treatment. Survivorship care strategies are recommended as crucial tool to assist communication and distribution of responsibility during the course of transition from active therapeutics to survivorship care. This care plan directs recommendations for the type and timing of follow-up imaging, laboratory tests, and other office visits. In addition cancer survival plan should incorporate necessary information regarding the deleterious effects of chemotherapy and what to watch for specifically based on the type of cancer and treatment received. Ideally, the oncology team should also work with the patient to develop an individualized cancer survivorship care plan for breast cancer survivors. A brief outline of a patient's diagnosis and treatment received should be presented by the oncologist team when a patient with breast cancer transitions care to other providers; a treatment outline should explain the type and stage/side of the cancer, type of surgery, the name of the chemotherapy/hormones/biologics and collective doses of chemotherapy, and the types and collective doses of radiation therapy, together with the fields and extent of the radiation. However, the field of oncology in context with cancer survivorship and care plan is mostly struggling with how to excellently meet this recommendation and in categorize the

specific benefits of such care plans. Various tools and strategies to assist the creation and distribution of these care plans are being actively considered for all tumor sites, including breast cancer. Currently, challenges in workflow and tools make this difficult, but the field is working toward a sustainable solution. Patients can start the building of a cancer survivorship care plan course on the ASCO website (cancer.net/survivorship/follow-care-after-cancer-treatment/asco-cancer-treatment-and-survivorship-care-plans; at journeyforward.org/or livestrongcareplan.org/).

8. Conclusion and future perspective

Despite the increasing number and longevity of breast cancer survivors the symptomology associated to therapeutics can be burdensome, multifaceted and long lasting and usually undertreated. Recent data suggest that management symptomology of these survivors could improvement of QoL, while evidence about its ability to reduce healthcare utilization is scarce. Cancer survivorship programmes help breast cancer survivors organize and remember their appointments. Communication and cooperation among providers and survivors are critical in the management and survivorship of breast cancer patients after treatment. Oncology teams all are working to develop a cancer survivorship care plan for breast cancer survivors. This care plan should guide survivors about the future laboratory tests, cardiovascular diseases, musculoskeletal issues, psychosocial issues, and other issues from which they usually suffer after treatment. The care plan should include information on the risk for late effects of treatment. To summarize, cancer survivorship care is need of a hour to allow better care for a larger number of breast cancer survivors in a financially sustainable manner.

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Conflict of interest

The authors declare no conflicts of interest.

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Chapter 3

Multiscale Stochastic Modeling Connects Cancer Drug Resistance Mechanisms to Population Survival Rates

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Additional information is available at the end of the chapter

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Abstract

Drug resistance significantly limits the long-term effectiveness of targeted therapeutics for cancer patients. Recent experimental studies have demonstrated that cancer cell heterogeneity and microenvironment adaptations to targeted therapy play important roles in promoting the rapid acquisition of drug resistance and in increasing cancer metastasis. The systematic development of effective therapeutics to overcome drug resistance mechanisms poses a major challenge. In this study, we used a modeling approach to connect cellular mechanisms underlying cancer drug resistance to population-level patient survival. To predict progression-free survival in cancer patients with metastatic melanoma, we developed a set of stochastic differential equations to describe the dynamics of heterogeneous cell populations while taking into account micro-environment adaptations. Clinical data on survival and circulating tumor cell DNA (ctDNA) concentrations were used to confirm the effectiveness of our model. Moreover, our model predicted distinct patterns of dose-dependent synergy when evaluating a combination of BRAF and MEK inhibitors versus a combination of BRAF and PI3K inhibitors. These predictions were consistent with the findings in previously reported studies. The impact of the drug metabolism rate on patient survival was also discussed. The proposed model might facilitate the quantitative evaluation and optimization of combination therapeutics and cancer clinical trial design.

Keywords: cancer drug resistance, population survival, mathematical modeling

1. Introduction

Drug resistance places an often inevitable limit on the long-term effectiveness of targeted therapeutics for cancer patients [1, 2]. Considerable efforts have been made to combat drug resistance and improve patient survival. Although the underlying molecular and cellular

mechanisms are complex, some paradigms of drug resistance mechanisms have been established [3–8].

It is widely acknowledged that the inherent heterogeneity [9, 10] of cancer cell populations, which is assumed containing both drug-sensitive and drug-resistant cells, contributes to drug resistance and metastasis [11–14]. A recent study [15] revealed a novel drug resistance mechanism in which drug-sensitive cancer cells secrete various soluble factors (e.g., IGF and HGF) into the tumor microenvironment in response to targeted therapy. These secreted factors can promote the growth, dissemination and metastasis of drug-resistant cancer cells and support the survival of drug-sensitive cells. Therefore, microenvironment adaptation [16] plays an important role in the rapid emergence of acquired drug resistance.

Evaluating cancer therapeutics in the context of tumor heterogeneity and microenvironment adaptation is very complex. In traditional *in vitro* and *in vivo* experiments, multiple cell types and multiple drug dosages must be considered, in addition to other experimental conditions and challenges in human population studies. As such, these studies are expensive and time consuming. Therefore the systematic development of effective therapeutics to overcome drug-resistance mechanisms has posed a major challenge. Mathematical modeling may potentially serve to bridge molecular/cellular mechanisms of drug resistance and population-level patient survival, and facilitates the quantitative evaluation and optimization of combination therapeutics and cancer clinical trial design.

Many mathematical and computational models have been developed to simulate tumor growth and drug response. For example, the cellular automata model [17, 18] or agent-based model [19–21], continuum partial differential equations model [22, 23] and hybrid discrete-continuum model [24, 25] have all been applied to evaluate tumor growth at the molecular, cellular and/or tissue level. These models have substantially advanced our understanding of tumor initiation and progression. However, due to their complexity and/or intensive computing burden, these models have rarely been applied to predict population-scale patient survival. Haeno et al. [26] developed a mathematical framework to describe pancreatic metastasis using a branching process to help understand cancer growth dynamics during metastasis and identify optimal therapeutic interventions. However, this framework focused on genetic mutation-induced drug resistance and did not address the role of targeted therapy-induced microenvironment adaptations in drug resistance. The use of combination therapy has been suggested in cases of drug resistance, such as in advanced melanoma patients with BRAF mutations [15, 16]. Therefore, the development of mathematical models capable of quantitatively evaluating synergism in combination drug therapy is desirable.

In this study, we created a multiscale model comprising a set of stochastic differential equations driven by both the Wiener process and Poisson process to describe pharmacokinetics, cellular dynamics, and progression-free survival at the patient level while accounting for microenvironment adaptations (**Figure 1**). Our model was subsequently verified using population- and cellular-scale clinical data. Then, we evaluated the efficiency and synergy of different combination therapies (combinations of BRAF, MEK and PI3K inhibitors). Our modeling revealed that different patterns of synergy existed for these combinations. Finally, sensitivity analysis revealed several key parameters that may combine with each other to affect the cancer cellular dynamics and patient survival, and facilitates the quantitative evaluation and design

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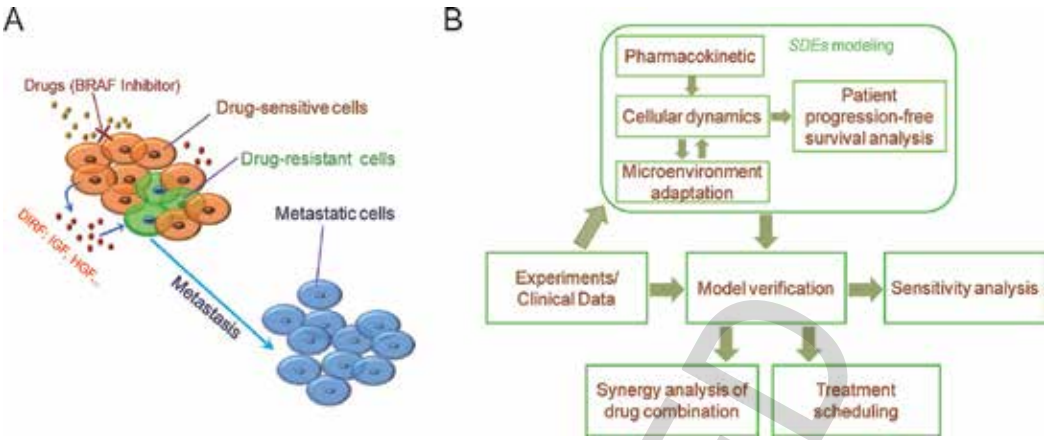

Figure 1. (A) Schematic representation of therapy-induced drug resistance and metastasis. Targeted therapy (e.g., treatment of melanoma using BRAF kinase inhibitors) is effective on drug-sensitive cells; however, a small number of pre-existing drug-resistant cancer cells are unaffected by treatment. In response to drug treatment, drug-sensitive cancer cells secrete various compounds (e.g., IGF and HGF) into the tumor microenvironment. These compounds were termed drug-induced resistance factors (DIRFs) in this study. The secreted DIRFs enhance the growth, dissemination and metastasis of cancer cells [15]. In our mathematical model, the metastatic cells refer to the new metastatic cells after the initiation of drug treatment. (B) A flowchart of our work. We constructed a stochastic model comprised of a set of stochastic differential equations driven by both Wiener and Poisson processes to model pharmacokinetics, DIRF secretion and cellular dynamics based on the recent experiments and clinical data. This enabled us to calculate progression-free survival *in silico*. Our model was then verified by comparing its predictions against clinical patient survival data. We used the model to quantitatively evaluate the efficiency and synergy of two combination therapies (BRAF inhibitor plus MEK inhibitor and BRAF inhibitor plus PI3K inhibitor). Furthermore, sensitivity analysis revealed several important parameters in the model that may provide implications for the design of combination therapies. In addition, we also examined cellular- and patient-level responses to different drug treatment schedules and investigated the impact of heterogeneity in drug metabolism rates on patient survival.

of combination therapeutics. In addition, we examined the impacts of different treatment schedules and drug metabolism rates on patient survival.

2. Models

2.1. Cellular dynamics

Tumors are heterogeneous (e.g., in their mutation profiles), resulting in some tumor cells possessing sensitivity to drug therapy and others in the same tumor exhibiting resistance to it. To model growth, transition and dissemination dynamics in drug-sensitive and drug-resistant cancer cells in patients with metastatic disease, we employed the following set of stochastic differential equations (SDEs):

$$dC_S = \overbrace{\tilde{r}_S \cdot C_S \cdot \left(1 - \frac{C_S + C_R}{T_{\max}}\right) dt}^{Growth} - \overbrace{u \cdot C_S dt}^{Transition} - \overbrace{\tilde{d}_S \cdot C_S dt}^{Death} + \overbrace{\sigma_1 C_S dW_1}^{Diffusion} - \overbrace{q_M \cdot C_K \cdot C_S \cdot dN_t}^{Dissemination} \quad (1)$$

$$dC_R = \overbrace{\tilde{r}_R \cdot C_R \cdot \left(1 - \frac{C_S + C_R}{T_{\max}}\right) dt}^{Growth} + \overbrace{u \cdot C_S dt}^{Transition} + \overbrace{\sigma_2 C_R dW_2}^{Diffusion} - \overbrace{\tilde{q}_M \cdot C_K \cdot C_R \cdot dN_t}^{Dissemination} \quad (2)$$

where C_S and C_R represent relative numbers (assumed in the unit of 10^8 [26–28]) of drug-sensitive cancer cells and drug-resistant cancer cells, respectively. The first terms on the right-hand side of Eqs. (1) and (2) describe the growth of sensitive cells and resistant cells, respectively. $\tilde{r}_S$ and $\tilde{r}_R$ are growth rate coefficients associated with these two cell types. The growth of drug-sensitive and drug-resistant tumor cells was assumed to follow a logistic growth law [29, 30]. $T_{\max}$ represents maximal carrying capacity. The second terms in equation (1 and 2) describe the transition from sensitive cells to resistant cells, e.g., due to genetic/epigenetic mutations. u represents the mutation rate in drug-sensitive cells as they convert to drug-resistant cells (i.e., mutation-driven drug resistance). The third term in Eq. (1) describes the drug-induced death of drug-sensitive cells. $\tilde{d}_S$ is the death rate of drug-sensitive tumor cells following treatment (e.g., BRAF inhibitors for V600 mutated melanoma) and depends on drug concentration (D) via the equation $\tilde{d}_S = d_S \cdot D/(K_{Drug} + D)$, where d_S represents maximal death rate, and K_{Drug} is a Michaelis constant representing the drug concentration associated with reaching the half-maximal inhibition effect. The fourth term (also called a diffusion term) in Eq. (1) simulates the stochastic fluctuation of cell numbers and is modeled by the standard Wiener process W that is described as

$$\Delta W = W(t + \Delta t) - W(t) \sim N(0, \Delta t) = \sqrt{\Delta t} N(0, 1) \tag{3}$$

where $N(0,1)$ is a unit normal distribution. The third term in Eq. (2) is similar. σ_i (i = 1, 2) represents the diffusion rate. The last terms in equation (1-2) describe the dissemination of existing cancer cells.

Independently of W_i, N_t represents a Poisson process with intensity λ and describes the count of metastasis within a cancer cell population [31, 32]. Specifically, the Poisson process N_t ($t \geq 0$) is characterized by

$$P[N_{t+\tau} - N_t = k] = \frac{e^{-\lambda\tau}(\lambda\tau)^k}{k!}, \quad k = 0, 1, \cdots, \tag{4}$$

where λ is the expectation of disseminating cell number within per unit time (Day). In addition, N_t has independent increments, and $N_0 = 0$. In the above equations (1 and 2), both drug-sensitive and drug-resistant cancer cells were assumed to have the potential to further metastasize. q_M and $\tilde{q}_M$ represent the dissemination rates of drug-sensitive and drug-resistant cells, respectively. $\tilde{q}_M$ is regulated by drug-induced resistance factors as described below. It should be noted that the metastasized cells in patients before therapy were considered to be included in these sensitive or resistant cells, and a new variable was introduced to account for new metastasis after the initiation of targeted therapy as follows.

Therapy-induced drug resistance can intensify tumor metastasis [15, 16]. The growth of new metastatic tumor cells following the drug treatment was modeled using a SDE driven by a jump process as follows:

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$$dC_M = \overbrace{r_M \cdot C_M \cdot \left(1 - \frac{C_M}{M_{\max}}\right) dt}^{Growth} + \overbrace{\sigma_4 C_M dW_4}^{Diffusion} + \overbrace{q_M \cdot C_K \cdot \left(1 - \tilde{d}_S\right) C_S \cdot dN_t}^{Metastasis\ from\ sensitive\ cells} + \overbrace{\tilde{q}_M \cdot C_K \cdot C_R \cdot dN_t}^{Metastasis\ from\ resistant\ cells} + \overbrace{\tilde{q}_M \cdot C_K \cdot C_M \cdot dN_t}^{Second\ metastasis} \quad (5)$$

where C_M represents the number of new metastatic cells after the initiation of new therapy. The first term in Eq. (3) describes the growth of the metastatic cells, and r_M is a metastatic cell growth rate coefficient. $M_{\max}$ is the maximal carrying capacity of metastatic cell growth. The second term (diffusion term) simulates fluctuation of metastatic cell population as mentioned above. Metastasis from existing cancer and metastatic emissions by the metastases themselves (i.e., secondary metastasis) [33] were taken into account, which were modeled in the last three terms of Eq. (3). q_M and $\tilde{q}_M$ respectively represent dissemination rates of drug-sensitive and drug-resistant cancer cells as described above. Metastatic rates were assumed to depend on existing tumor size (i.e. drug-sensitive/resistant cancer cell numbers C_S and C_R) [34] and angiogenic cell number (C_K) [29]. The drug effect on drug-sensitive metastatic cells was incorporated by using $\left(1 - \tilde{d}_S\right) C_S$ in the third term. By assuming that newly developed metastasis sites are more supportive of the growth of invasive cancer cells, a positive net increase rate of new metastatic cells due to the secondary metastasis was introduced in the last term of the above equation.

Angiogenic growth in tumors is induced by the secretion of angiogenic growth factors (e.g., VEGF). We modeled angiogenesis based on previously established work [35] with the following equation:

$$dC_K = \overbrace{\tilde{r}_K \cdot (C_S + C_R) \cdot \left(1 - \frac{C_K}{K_{\max}}\right) dt}^{Growth} - \overbrace{d_K \cdot (C_S + C_R)^{2/3} \cdot C_K dt}^{Inhibition} + \overbrace{\sigma_3 C_K dW_3}^{Diffusion} \quad (6)$$

where C_K represents the number of angiogenic cells. The first term describes the growth of angiogenic cells induced by tumor cells. $\tilde{r}_K$ is a growth rate coefficient associated with angiogenic cells, and $K_{\max}$ is the maximal carrying capacity for blood vessel growth. The second term describes the growth inhibition of angiogenic cells by tumor cells with a coefficient d_K. Newly grown blood vessels can provide tumor cells with nutrients (such as oxygen and glucose) and thus influence the maximal carrying capacity of tumor cells [29, 30] as follows: $T_{\max} = C_K/(1 + C_K/K_{Tm})$, where K_{Tm} is a Michaelis constant.

2.2. Pharmacokinetics

Pharmacokinetics describe the dynamics of drug absorption, metabolism and elimination by the body [36]. These processes are often modeled as follows [37, 38]:

$$dD = -\overbrace{d_{drug} \cdot Ddt}^{Elimination} + \overbrace{U(t)dt}^{Delivery} + \overbrace{\sigma_5 DdW_5}^{Diffusion} \tag{7}$$

where D represents drug concentration in the body. The first term in the above equation models the first-order elimination rate of drugs, and d_{drug} is a metabolic rate coefficient of patients. $U(t)$ in the second term is the rate of drug delivery. Brownian motion was also assumed in the above equation to accommodate stochasticity [37, 38].

The initial conditions of the Eqs. (1–2, 5, 6) were set to C_S = 0.2, C_R = 0.001 and C_K = 0.1, simulating the relative cell number (in the unit of 10^8 [26–28]) in patients (e.g., patients with metastatic melanoma and BRAF V600 mutations) before initiation of the new therapy. Starting from the initiation of the drug treatment, the number of new metastatic cells was counted, with an initial value C_M = 0. The initial concentration of drug was set to 0. The uniqueness of the solution to the above SDEs (Eqs. (1–7)) were easily obtained, since their coefficients satisfies the appropriate growth conditions and are locally Lipschitz continuous [39]. We employed a time-adapted Euler scheme [40] to provide numerical solutions to the SDEs driven by both diffusion (Brownian motion) and jump (Poisson process).

2.3. Microenvironment adaptations to drug treatment

As demonstrated by recent experimental and preclinical studies [15], when BRAF inhibitors (BRAF-I), such as Vemurafenib and Dabrafenib, are administered to cancer patients with BRAF mutations, they can induce drug-sensitive cancer cells to secrete resistance factors (e.g., IGF and HGF) into the tumor microenvironment. We modeled the secretion of drug-induced resistance factors (DIRFs) by drug-sensitive tumor cells according to Michaelis–Menten kinetics [41] as follows:

$$\frac{d[DIRF]}{dt} = \overbrace{\frac{V_{DS} \cdot D}{K_{DS} + D} \cdot C_S}^{Secretion} - \overbrace{d_{DIRF} \cdot [DIRF]}^{Degradation} \tag{8}$$

where V_{DS} is the maximal secretion rate of DIRFs from drug-sensitive cells, and K_{DS} is a Michaelis constant representing the drug concentration at which a half-maximal secretion rate is achieved. d_{DIRF} represents DIRF degradation rate.

It was assumed that DIRF secretion and/or degradation in the microenvironment are much faster processes than cellular phenotype switching and shifts in cellular population dynamics. Therefore, using a quasi-steady state assumption we can express the secreted DIRF concentration as follows:

$$[DIRF] = \frac{1}{d_{DIRF}} \cdot \frac{V_{DS} \cdot D}{K_{DS} + D} \cdot C_S = \tilde{V}_{DS} \cdot \frac{D}{K_{DS} + D} \cdot C_S \tag{9}$$

where $\tilde{V}_{DS} = V_{DS}/d_{DIRF}$.

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Secreted DIRFs can promote outgrowth, dissemination and metastasis in drug-resistant cells and enhance survival in drug-sensitive cells [15, 16]. The effects of DIRFs on cellular dynamics were modeled using the following functions, which correlate DIRF concentration to the growth, dissemination and metastasis rates of three types of cancer cells:

$$\tilde{r}_S = r_S \cdot \left(1 + \frac{[DIRF]}{K_{1\_DIRF} + [DIRF]}\right) \tag{10}$$

$$\tilde{r}_R = r_R \cdot \left(1 + \frac{[DIRF]}{K_{2\_DIRF} + [DIRF]}\right) \tag{11}$$

$$\tilde{q}_M = q_M \cdot \left(\alpha + \frac{[DIRF]}{K_{3\_DIRF} + [DIRF]}\right) \tag{12}$$

$$\tilde{\lambda} = \lambda \cdot \frac{[DIRF]}{K_\lambda + [DIRF]} \tag{13}$$

Eq. (8) describes the dependence of growth rate of sensitive cells on the secreted DIRF concentration, where r_S is a basal growth rate of sensitive cells, and K_{1_DIRF} is a Michaelis constant of DIRF for regulating r_S. Eq. (9) depicts the growth rate of resistant cells depending on the secreted DIRF concentration, where r_R is the basal growth rate of resistant cells, and K_{2_DIRF} is a Michaelis constant of DIRF for regulating r_R. Eq. (10) correlates DIRF concentration to the dissemination rate of cancer cells, where α is the regulatory coefficient, and K_{3_DIRF} is a Michaelis constant of DIRF for increasing q_M. Eq. (11) represents the metastasis rate regulated by DIRF concentration with K_λ being a Michaelis constant.

In this way, we linked the short-term timescale (minutes) associated with intercellular signaling to the long-term timescale (days) necessary for cellular dynamics [42–44].

2.4. Progression-free survival analysis of a patient population

Cancer progression is often clinically evaluated using radiographic imaging. In the below analysis, if a patient's total tumor cell number or tumor volume exceeded a pre-set threshold, C_{Th} (assumed to be 1.6 in this work), then we considered the patient's cancer to be progressing. Therefore, progression-free survival (PFS) time (T_{PFS}) was defined as the length of time between initiation of therapy (t = 0, the starting time in our model) and initiation of cancer progression or death as follows:

$$T_{PFS} = \inf\{t : C_s(t) + C_R(t) + C_M(t) \geq C_{Th}\} \tag{14}$$

where T_{PFS} is a random variable due to the stochastic nature of cancer progression. In our simulations, N, which represents number of patients, was set to 100. We calculated the progression-free survival time for each patient in the simulation and then computed overall survival percentages and survival frequencies for the entire patient population under different treatment schedules. In the following text, patient survival refers to progression-free survival unless stated otherwise.

2.5. Incorporation of the effects of MEK and PI3K inhibitors into the model

Currently, in addition to BRAF inhibitors (e.g., Vemurafenib and Dabrafenib), several other targeted inhibitors, such as MEK inhibitors (e.g., Trametinib and Cobimetinib) and PI3K inhibitors (e.g., BEZ235) are in clinical trials for melanoma cancer patients. In this study, we investigated the synergy between BRAF inhibitors in conjunction with each of these other two inhibitor types by incorporating their effects into our model based on their different signaling mechanisms, respectively.

MEK is a downstream effector protein of RAF signaling [45]; therefore, we assumed that MEK inhibitors would produce similar effects to BRAF inhibitors when inducing the death of drug-sensitive cancer cells. It has been shown that both BRAF inhibitors and MEK inhibitors can increase the death rate of drug-sensitive cells. MEK inhibitors can also promote the secretion of drug-induced resistance factors (DIRFs) from drug-sensitive cells [15] because RAF and MEK share the same downstream effector, transcription factor FRA1, which has been identified as a major regulatory factor of DIRF secretion. Therefore, the effects of MEK inhibitor use were incorporated into the model using Hill functions as in Refs. [46, 47]:

$$\tilde{d}_S = d_S \cdot \left(\frac{BRAFi}{K_{BRAFi} + BRAFi} + \frac{MEKi}{K_{MEKi} + MEKi} \right) \tag{15}$$

$$[\mathrm{DIRF}] = \tilde{V}_{DS} \cdot \left(\frac{BRAFi}{K_{DS1} + BRAFi} + \frac{MEKi}{K_{DS2} + MEKi} \right) \cdot C_S \tag{16}$$

where d_S represents basal death rate. K_{BRAFi} and K_{MEKi} are Michaelis constants representing the BRAF inhibitor and MEK inhibitor concentrations at which half-maximal inhibition effects are reached. K_{DS1} and K_{DS2} are also BRAF inhibitor and MEK inhibitor Michaelis constants for DIRF secretion. C_S is the number of drug-sensitive cells.

In drug-resistant cells, the PI3-Kinase (PI3K)/AKT pathway is over-activated by DIRFs [15]; therefore, a PI3K inhibitor may repress DIRF-stimulated PI3K/AKT pathway activation and thus reduce DIRF effects. We used an inhibition Hill function [47] to include the effects of PI3K inhibitor-mediated DIRF signal modification into our model:

$$[DIRF]_{Eff} = [DIRF]_{BRAFi} \cdot \frac{K_{PI3Ki}}{K_{PI3Ki} + PI3Ki} \tag{17}$$

where $[DIRF]_{Eff}$ represents effective action of DIRF inhibited by PI3K inhibitor, and $[DIRF]_{BRAFi}$ is the DIRF steady-state concentration following stimulation with a BRAF inhibitor. K_{PI3Ki} is the Michaelis constant for the PI3K inhibitor's half-maximal inhibition concentration. In this simplified way, we incorporated the effects of PI3K inhibitor into the model without considering complex intracellular signaling networks. This strategy enabled us to reduce the complexity of the model.

It should be noted that we used dimensionless concentrations of BRAF inhibitor, MEK inhibitor and PI3K inhibitor in the simulation by respectively normalizing them to the Michaelis constants

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K_{BRAFi}, K_{MEKi} and K_{PI3Ki}, as in our previous study [47]. As such, we did not introduce any additional parameters into the model to further reduce the number of unknown parameters.

2.6. Parameter estimation

Biological descriptions and values of the model parameters are listed in **Table 1**. Most of the parameters involved in the cellular dynamics and pharmacokinetics modules were collected from previous studies [26, 35, 37], while other parameters were calibrated according to recent experimental [15, 16] and clinical data [48]. We provide details regarding the calibration of the parameter values below.

2.6.1. Parameters involved in DIRF secretion

Recent studies [15] (extended data, Figure 4d in Ref. [15]) have reported that the levels of some murine stroma-derived cytokines, such as HGF, IGF, MFGE8 and TREM1, were upregulated by 1.6- to 10-fold in response to treatment with Vemurafenib (a BRAF inhibitor) compared to vehicle treatment. The newly secreted DIRFs are described in Eq. (7) in the main text. The DIRF basal level was assumed to be 0.1. To ensure that the change in total DIRF (basal plus newly secreted DIRFs) remained within a range of 1 to 10 according to the experiments mentioned above, $\tilde{V}_{DS}$ and K_{DS1} ($i = 1, 2$) were set to 100 and 10, respectively, as the concentration of BRAF inhibitor changed from 0.1 to 1 in the simulation. Note that in $\tilde{V}_{DS} = V_{DS}/d_{DIRF}$, the degradation rate ($d_{DIRF}$) is assumed to be 0.01 per day. Thus, the maximal DIRF secretion rate from drug-sensitive cells (V_{DS}) was accordingly set to 1.

2.6.2. Parameters involved in drug-resistant cell growth

The experimental data in Ref. [15] also indicated that the relative number of drug-resistant cancer cells in drug-sensitive tumors increased by approximately 2- to 4-fold after 3 days of drug treatment (Vemurafenib, Crizotinib and Erlotinib). The growth rate coefficient corresponding to drug-resistant cells regulated by DIRFs is described in Eq. (9) in the main text. Because the minimal and maximal DIRF concentrations were set to 0.1 and 0.9, as stated in the above section, α and K_{2_DIRF} were set to 0.1 and 0.3, respectively, to ensure an approximate 2- to 4-fold change in the relative number of drug-resistant cancer cells induced by the drug treatment on day 3, in agreement with the above-mentioned experimental data. The value of K_{1_DIRF}, the DIRF-associated Michaelis constant, was set in a similar way for drug-sensitive cancer cells.

2.6.3. Parameters involved in metastasis

The drug-resistant cancer cell dissemination rate coefficient that was regulated by DIRFs is described by Eq. (10) in the main text. We set the values of α and K_{3_DIRF} based on experimental data [15] (Figure 2D in Ref. [15]). The relative migration of drug-resistant cancer cells increased by 2- to 3-fold following BRAF inhibitor treatment compared to vehicle treatment. Therefore, to reproduce this change in our simulation, α was set to 0.3, and K_{3_DIRF} was set to 0.01. λ (intensity of Poisson process) in Eq. (11) was scaled and estimated from Ref. [26].

Symbol	Value	Unit	Description	Reference
r_S	0.192	Day^{-1}	Growth rate coefficient of drug-sensitive cells	[35]
r_R	0.192	Day^{-1}	Growth rate coefficient of drug-resistant cells	[35]
r_M	0.192	Day^{-1}	Growth rate coefficient of metastatic cells	[35]
r_K	0.01	Day^{-1}	Growth rate coefficient of angiogenic cells	Scaled from [35]
d_K	0.00873	Day^{-1}	Death rate coefficient of angiogenic cells	Scaled from [35]
q_M	$1*10^{-4}$	_	Dissemination rate of metastatic cells	Scaled from [26]
u	$4*10^{-5}$	_	Mutation rate of sensitive cells to resistant cells	[68]
d_S	0.366	Day^{-1}	Maximal inhibition rate of drug on drug-sensitive cells	Calibrated to [48]
d_{DIRF}	0.01	Day^{-1}	DIRF degradation rate	Estimated from [15]
V_{DS}	1	Day^{-1}	Maximal DIRF secretion rate from drug-sensitive cells	Estimated from [15]
K_{DS}	10	_	Michaelis constant of DIRF secretion	Estimated from [15]
λ	0.02	_	Intensity of Poisson process	Estimated from [26]
K_λ	0.2	_	Michaelis constant of DIRF secretion for Poisson intensity	Estimated from [26]
d_{drug}	0.04	Day^{-1}	Elimination rate constant of drug	Scaled form [37]
K_{BRAFi}	0.5	_	Michaelis constant of BRAF inhibitor	Assumed
K_{MEKi}	0.1	_	Michaelis constant of MEK inhibitor	Assumed
K_{PI3Ki}	0.1	_	Michaelis constant of PI3K inhibitor	Assumed
K_{1_DIRF}	0.3	_	Michaelis constant of DIRF for r_S	Estimated from [15]
K_{2_DIRF}	0.3	_	Michaelis constant of DIRF for r_R	Estimated from [15]
K_{3_DIRF}	0.1	_	Michaelis constant of DIRF for q_M	Estimated from [15]
α	0.01	_	Regulatory coefficient	Estimated from [15]
C_{Th}	1.6	10^8	Critical size of total tumor cells detected as progression	Assumed
K_{Tm}	10	_	Michaelis constants for maximal carrying capacity of drug-sensitive/resistant cells	Calibrated to [48]
M_{max}	1.67	10^8	Maximal carrying capacity of new metastatic cells	Calibrated to [48]
σ	0.02	_	Diffusion rate	Calibrated to [48]
C_{S0}	0.2	10^8	Initial value of the density of drug-sensitive cells	Assumed
C_{R0}	0.001	10^8	Initial value of the density of drug-resistant cells	Assumed
C_{K0}	0.1	10^8	Initial value of the density of angiogenic cells	Assumed
C_{M0}	0	10^8	Initial value of the density of new metastatic cells	Assumed

Each parameter is listed with its symbol, value, biological description and reference.
Remarks: "_" in the above **Table** 1 represents dimensionless unit.

Table 1. Values of parameters in the model.

2.6.4. Parameters fitted to the clinical data

The clinical data of progression-free survival percentages (Figure 1A in Ref. [48]) were used to estimate parameters including d_S (maximal inhibition rate of drug on drug-sensitive cells), K_{Tm}

(Michaelis constant for maximal carrying capacity of drug-sensitive/resistant cells), M_{max} (maximal carrying capacity of new metastatic cells) and σ (diffusion coefficient in Wiener process). The clinical data include progression-free survival percentages of a patient cohort treated with daily dosing of BRAF inhibitor vemurafenib and MEK inhibitor cobimetinib administered for 21 days, followed by 7 days off. By iteratively calibration, we chose the values of the above parameters which produced good agreement with the clinical data (**Figure 2**).

2.7. Sensitivity analysis

Sensitivity analysis was used to quantitatively explore the critical parameters in the model that affected cellular dynamics and survival percentage. A sensitivity coefficient [49] for total tumor cell number with respect to parameter p_j was calculated as follows:

$$S_j^T = \frac{\int_0^T \overline{C}_T\left(t,\tilde{p}_j\right)dt - \int_0^T \overline{C}_T\left(t,p_j\right)dt}{\int_0^T \overline{C}_T\left(t,p_j\right)dt} \Bigg/ \frac{\Delta p_j}{p_j} \approx \frac{\sum_{i=1}^{L} \Delta\overline{C}_T(t_i)}{\sum_{i=1}^{L} \overline{C}_T(t_i)} \Bigg/ \frac{\Delta p_j}{p_j} \tag{18}$$

where $\{t_i, i = 1, \cdots L\}$ is an equal partition of $[0, T]$, with L = 360 and T = 360 days in the simulation. $\overline{C}\left(t,p_j\right)$ and $\overline{C}\left(t,\tilde{p}_j\right)$ represent the median total tumor cell numbers with parameter p_j and varied parameter $\tilde{p}_j$ at time t, respectively. $\Delta\overline{C}_T(t_i) = \overline{C}_T\left(t_i,\tilde{p}_j\right) - \overline{C}_T\left(t_i,p_j\right)$ is the change of median total tumor cell number with the parameter perturbation $\Delta p_j = \tilde{p}_j - p_j$ at time t_i (day). Then, we obtained the relative changes for the area under curve of total tumor cell number with respect to the examined parameters.

The sensitivity coefficient of the survival percentage with respect to parameter p_j was also calculated according to the following formula:

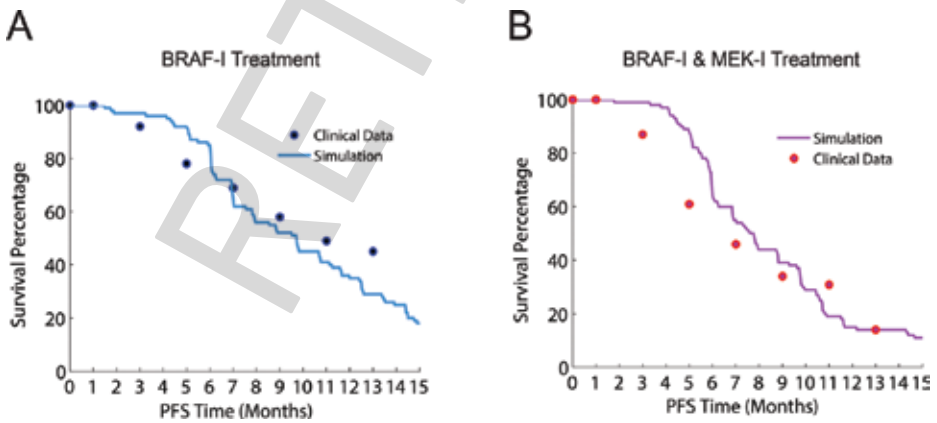

Figure 2. Fitting the model to the clinical data. Comparisons between the simulations and the clinical data [48] of patient survival percentages with (A) BRAF-I treatment and (B) BRAF-I&MEK-I treatment respectively. The mean squared relative error (MSRE) is 0.1910.

$$S_j^{SP} = \frac{\int_0^T SP\left(t, \tilde{p}_j\right)dt - \int_0^T SP\left(t, p_j\right)dt}{\int_0^T SP\left(t, p_j\right)dt} \Big/ \frac{\Delta p_j}{p_j} \approx \frac{\sum_{i=1}^{L} \Delta SP(t_i)}{\sum_{i=1}^{L} SP_T(t_i)} \Big/ \frac{\Delta p_j}{p_j} \tag{19}$$

where $SP\left(t, p_j\right)$ and $SP\left(t, \tilde{p}_j\right)$represent survival percentages with parameter p_j and varied parameter $\tilde{p}_j$ at time t respectively. $\Delta SP(t_i) = SP\left(t_i, \tilde{p}_j\right) - SP\left(t_i, p_j\right)$ is the change of survival percentage with the parameter perturbation $\Delta p_j = \tilde{p}_j - p_j$ at time t_i (day). Then, we obtained the relative changes for the area under curve of survival percentage with respect to the examined parameters.

2.7.1. Single-parameter sensitivity analysis

Each parameter was increased by 50% from its estimated value and we then obtained the relative changes in area under curve of total tumor cell number and area under survival curve, as defined by Eqs. (16) and (17). The computations were repeated 20 times, and the mean value and standard deviation of the sensitivity coefficients were calculated.

2.7.2. Two-parameter sensitivity analysis

To assess the combinatorial effects of parameters involved in the model, we performed a two-parameter sensitivity analysis. The values of each pair of two different parameters were increased by 50% from their original values simultaneously. All other parameters remained at their base values. Taking into account the stochasticity of the model, the computations were repeated 20 times, and the mean value of the sensitivity coefficients was calculated.

3. Results

3.1. *In silico* prediction of cellular kinetics and patient survival following drug treatment

We investigated cellular response kinetics following drug treatment *in silico*. In **Figure 3**, a typical simulation of BRAF-I treatment is shown. **Figure 3A** details BRAF-I kinetics for 100 cancer patients. The inhibitor was administered daily for 3 weeks followed by 1 week of no treatment, concordant with the drug schedule of a previous study [48]. **Figure 3B** and **2E** show the time courses of all 100 samples with respect to numbers of drug-sensitive cancer cells, drug-resistant cancer cells, metastatic cells and total tumor cells. Drug-sensitive cancer cell growth was repressed following drug administration, but it periodically rebounded during no treatment weeks. Interestingly, metastatic cell growth (**Figure 3D**) showed a similar pattern among the patients: an initial slow growth period (the length of which varied in different patients) followed by a rapid increase within ~1 month. The metastatic cell populations in different patients exhibited different transition times, resulting in heterogeneous sizes of cancer cells among patient population (**Figure 3E**). Interestingly, this "all-or-no" metastasis causes

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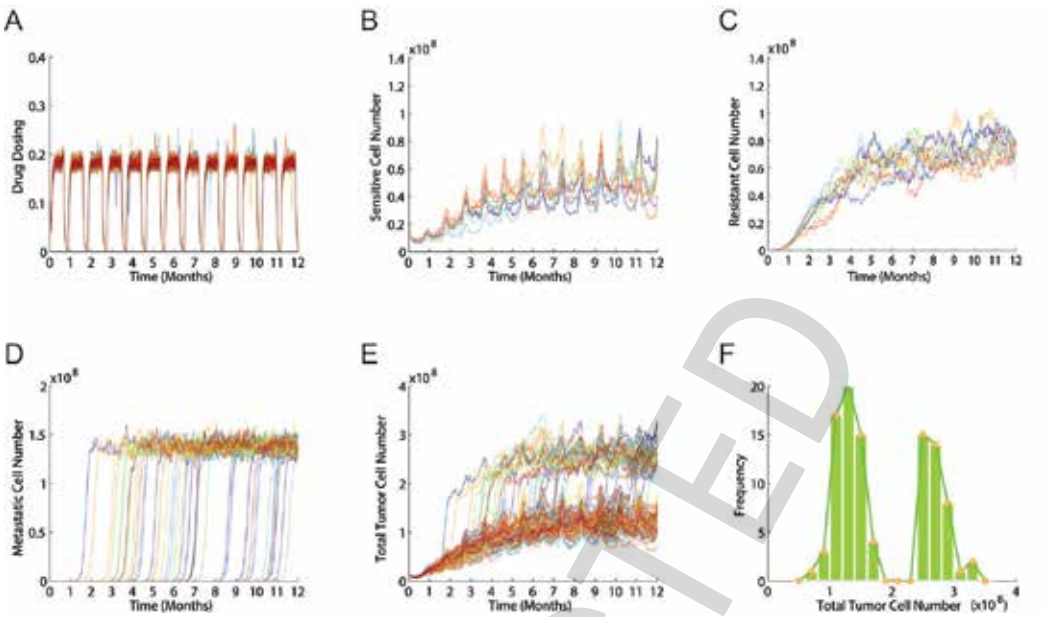

Figure 3. A typical simulation of cellular- and patient-level responses to drug treatment. A BRAF inhibitor was administered daily for 3 weeks, followed by 1 week off [48]. (A) Pharmacokinetics. Time courses of 100 samples showing numbers of (B) drug-sensitive cancer cells, (C) drug-resistant cancer cells, (D) new metastatic cells, and (E) total tumor cells. (F) Bimodal distribution of total tumor cell number at 360 days.

a bimodal distribution for the number of total tumor cells after 12 months (**Figure 3F**), indicating that cancer in some patients progressed but not yet in others.

For comparison, cellular dynamics without drug treatment are shown in **Figure 4**. In this case, the distribution (**Figure 4D**) of the number of total tumor cells was changed to an asymmetric bimodal distribution with decreased frequencies of cell numbers at high level (**Figure 4E**) due to the lack of the drug-induced metastasis (**Figure 4C**). We also applied our model to investigate the effects of targeted therapy on patient survival at the population level. **Figure 4F** shows the survival percentage of 100 cancer patients undergoing BRAF-I treatment from 0 to 360 days compared with no treatment controls. Our simulation demonstrated that treatment with BRAF-I significantly prolonged progression-free survival in melanoma patients harboring BRAF mutations. This result is consistent with clinical studies of melanoma patients harboring the BRAF V600E mutation [48, 50].

3.2. Validation of cellular kinetics and patient survival using clinical data

We next validated our model using clinical data. In **Figure 5**, a comparison of our model predictions with clinical population-scale survival data is shown [51]. The clinical data included distributions of progression-free survival times for 54 patients in each group treated either with Dabrafenib monotherapy (a BRAF-I) (**Figure 5A**) or with a combination of BRAF-I and Trametinib (a MEK inhibitor, or MEK-I) at doses of 150 and 1 mg (1X; **Figure 5B**) or 150 and 2 mg (2X; **Figure 5C**). To simulate the conditions used to produce the clinical data, our

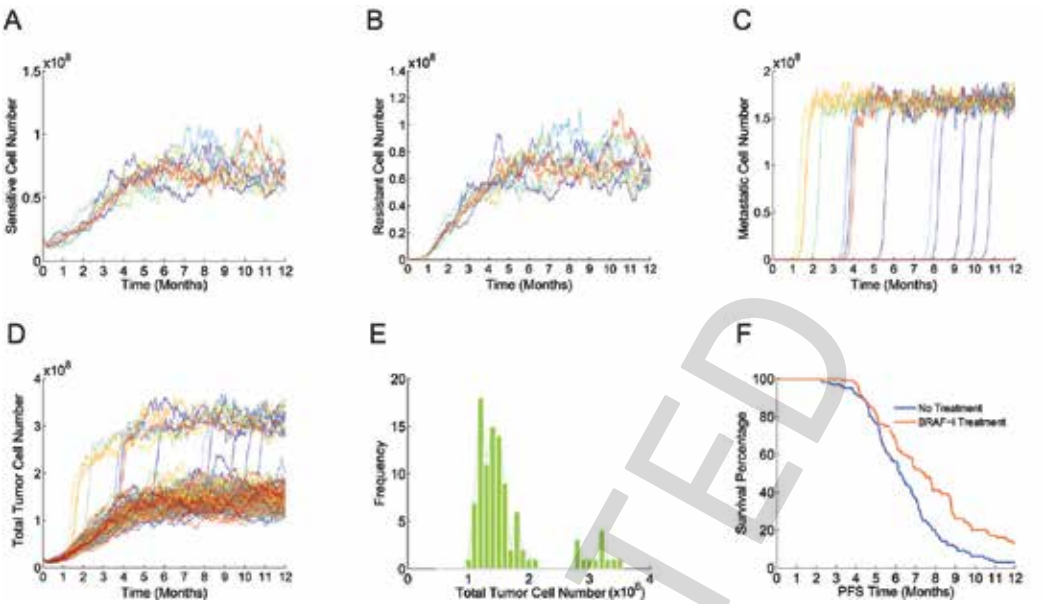

Figure 4. Cellular dynamics without drug treatment. Time courses of 10 samples showing relative numbers of (A) drug-sensitive cancer cells, (B) drug-resistant cancer cells, and 100 samples showing relative numbers of (C) new metastatic cells and (D) total tumor cells. (E) Distribution of total tumor cell number at 450 days. (F) Progression-free survival percentages of patients with BRAF-I treatment (red line) and without treatment (blue line).

model (red lines in **Figure 5A–C**) examined the following three treatment strategies: BRAF-I alone, BRAF-I combined with 1 mg MEK-I, and BRAF-I combined with 2 mg MEK-I. It should be noted that the drug doses in the simulation have been normalized (refer to the Model section). We computed progression-free survival times using our model and compared them against the clinical data; the predicted and experimental results were in good agreement. Furthermore, as shown in **Figure 5D**, our model predicted that the combination therapies enhanced progression-free survival more than the monotherapy, consistent with the clinical data.

Figure 6 shows a validation of the sudden increase observed in metastatic cell number in the model using clinical values of circulating tumor DNA (ctDNA). ctDNA has been proposed as a promising biomarker for monitoring metastatic cancers [52]. We used clinical data consisting of plasma ctDNA concentrations from nine patients [53] treated with BRAF-I and MEK-I in combination to verify the predicted metastatic cell growth. Of the nine evaluated patients, four showed elevated ctDNA levels while undergoing combination therapy. In **Figure 6A**, a comparison of the simulated growth pattern of metastatic cells with the clinical ctDNA data is shown. Both the clinical data and the model prediction showed a pattern of explosive metastatic cell growth in several patients. In addition, the model predicted that new metastatic cell numbers would either remain at an undetectable, low level (48%) or significantly increase (52%) (**Figure 6B**). A threshold of metastatic cell number was set to 1, separating these two distinct levels. This prediction agrees with the clinical plasma ctDNA concentrations, in which ctDNA levels are either undetectable (5/9) or elevated (4/9).

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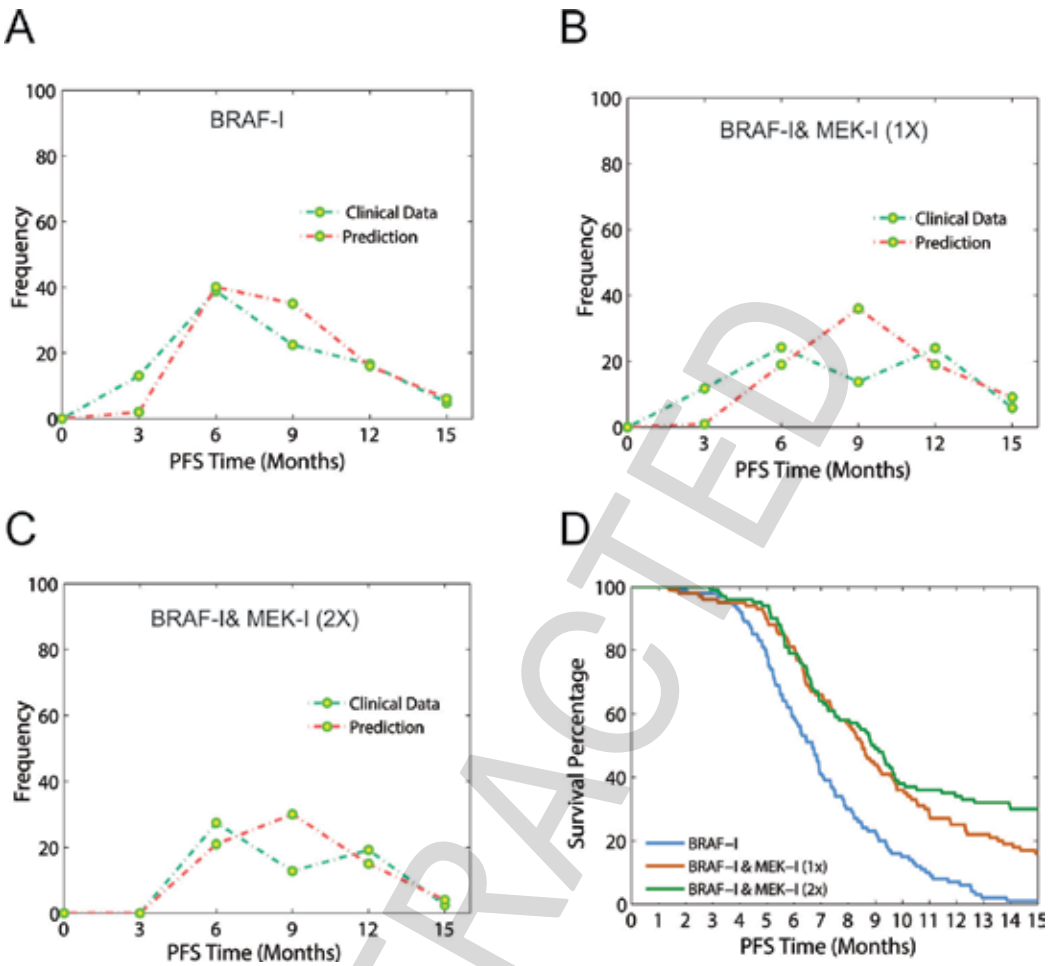

Figure 5. Validation of the model on the population scale by comparing survival frequency predictions with clinical survival data [51]. We compared progression-free survival (PFS) time determined by the model prediction to that calculated from actual clinical data of a patient cohort administered Dabrafenib (a BRAF inhibitor) monotherapy (A), a combination of 150 mg Dabrafenib and 1 mg Trametinib (a MEK inhibitor) (BRAF-I&MEK-I, 1X) (B), and a combination of 150 mg Dabrafenib and 2 mg Trametinib (BRAF-I&MEK-I, 2X) (C). In the clinical data, there are 54 patients for each treatment group. In our simulation, 100 patients were simulated for each group. (D) The predicted progression-free survival percentages showed improved survival over time following the administration of combination therapeutics compared to BRAF-I monotherapy. The predicted survival curve shown here has a highly similar pattern to that in Figure 1A of Ref. [51].

3.3. Evaluation of drug combination synergy

Currently, in addition to BRAF inhibitors (e.g., Vemurafenib and Dabrafenib), several other targeted inhibitors, including MEK inhibitors (e.g., Trametinib and Cobimetinib) and PI3K inhibitors (e.g., BEZ235), are being evaluated in clinical trials for treatment of melanoma patients. In the following study, we investigated whether the co-administration of BRAF-I with either MEK-I or PI3K inhibitor (PI3K-I) produces synergistic effects. To accomplish this, we

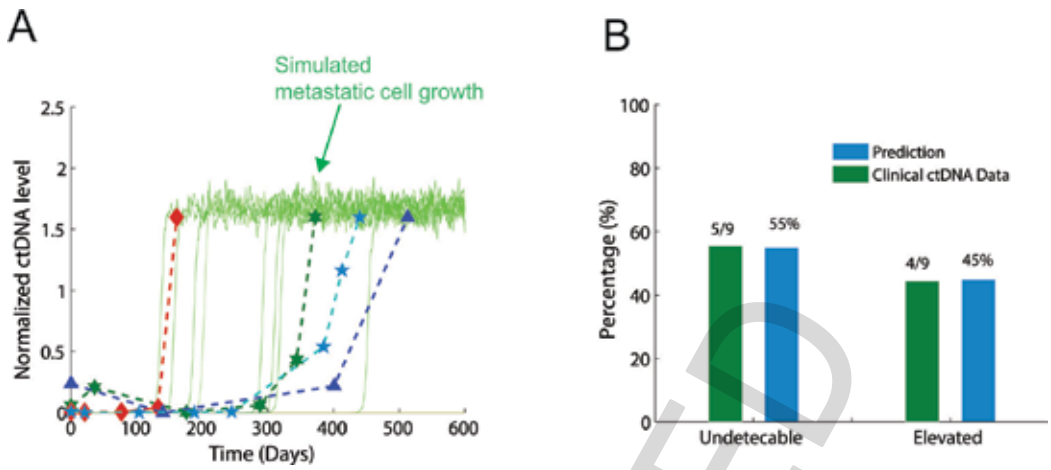

Figure 6. Validation of the model on the cellular scale by comparing metastatic cell growth patterns with normalized ctDNA data [53]. ctDNA concentrations were collected from nine patients treated with BRAF and MEK inhibitors (BRAF-I and MEK-I) in combination. Increased ctDNA levels were evident in four of the nine patients. These values were plotted against the predicted metastatic cell growth pattern for model validation. (A) Increased ctDNA concentrations in four patients co-treated with BRAF-I and MEK-I compared to simulated metastatic cell-growth curves. Both the ctDNA concentration data and the model prediction showed that some patients underwent an explosive growth pattern of metastatic cells. (B) Predicted distribution of the number of metastatic cells versus the clinical data.

incorporated the effects of these inhibitors into our model (see details in Model section) based on their specific signaling mechanisms.

A Bliss combination index [54, 55] was used to quantitatively evaluate the synergy produced by BRAF-I and MEK-I co-treatment and BRAF-I and PI3K-I co-treatment as follows:

$$CI(x_1, x_2) = R_{12}(x_1, x_2) - [R_1(x_1) + R_2(x_2) - R_1(x_1) \cdot R_2(x_2)] \tag{20}$$

where $R_i(x_i) = (\overline{C}_T - \overline{C}_T(x_i))/\overline{C}_T$ is the reduction of the median total tumor cell number ($\overline{C}_T$) induced by the single BRAF inhibitor ($i = 1$), or either MEK inhibitor or PI3K inhibitor ($i = 2$) alone at dose x_i.$R_1(x_1) + R_2(x_2) - R_1(x_1)R_2(x_2)$ in Eq. (18) is the expected effect of combination therapy, and $R_{12}(x, y)$ is the actual outcome produced by the combination. Hence, the combination of BRAF-I with MEK-I or PI3K-I is synergistic if $CI > 0$, antagonistic if $CI < 0$, and additive if $CI = 0$.

The model predicted that both the BRAF-I & MEK-I combination and the BRAF-I & PI3K-I combination possess dose-dependent synergy but in different patterns (**Figure 7**). The BRAF-I & MEK-I combination was synergistic at lower combined dosages, while the BRAF-I & PI3K-I combination exhibited greater synergy at higher dosages.

Importantly, the predicted differences in the synergies of these two combinations are consistent with experimental studies [56, 57], which have reported stronger synergy for BRAF-I & PI3K-I co-treatment than BRAF-I & MEK-I co-treatment. Our model also predicted that the maximal CI value for the BRAF-I & PI3K-I combination (up to 0.3, **Figure 7A**) is greater than that of the BRAF-I & MEK-I combination (less than 0.15, **Figure 7B**). This demonstrates good agreement

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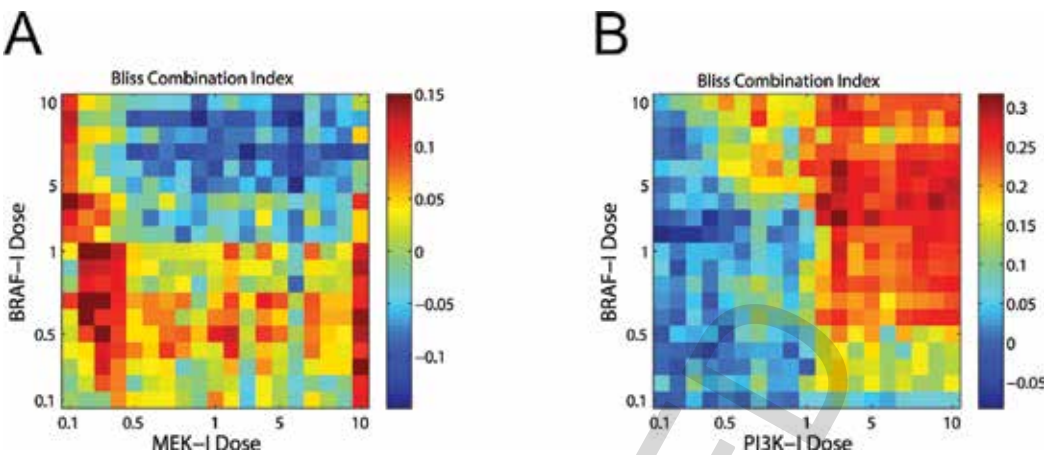

Figure 7. Evaluation of drug combinations for synergy using various concentrations of drugs. (A) co-administration of BRAF and MEK inhibitors led to dose-dependent synergy. (B) co-administration of BRAF and PI3K inhibitors led to dose-dependent synergy.

between our model's predictions and the experimental data. In addition, the dose-dependent synergy predicted by our model might also help explain contradictory experimental observations that different PI3K/AKT inhibitors (e.g., MK2206 and Perifosine) produce opposing effects when combined with BRAF-I (PLX4032) [58].

3.4. Sensitivity analysis

We conducted parameter sensitivity analysis to examine whether the model is robust to parameter variations and to identify parameters with critical effects on both cellular dynamics and patient survival. **Figure 8A**, **B** show a single-parameter sensitivity analysis. Here, a parameter was regarded to be sensitive/critical if its sensitivity coefficient is greater than 0.2. The results showed that the model was rather robust with respect to the variations of most parameters including those calibrated. Moreover, the following parameters were critical to model outputs: growth rate and death rate in angiogenic cells (r_K, d_K) and metastatic rate (λ). Since angiogenesis positively supports the sustained growth of the tumor cells and provides an avenue for dissemination and translocation of metastatic cancer cells (especially drug-resistant metastatic cells), therefore the growth/death rates in angiogenic cells as well as metastatic rate of cancer cells were shown to significantly affect cellular dynamics and patient survival.

We further conducted a two-parameter sensitivity analysis to investigate how the parameters combine with each other to affect cellular dynamics and patient survival. The values of each pair of two different parameters were increased by 50% from their original values simultaneously. The computations were repeated 20 times, and the mean value of the sensitivity coefficients was calculated. **Figure 8C**, **D** show the relative changes of the areas under the curves of the total tumor cell number (**Figure 8C**) and patient survival percentage (**Figure 8D**) with respect to the combinatorial variations in parameter values. This global sensitivity

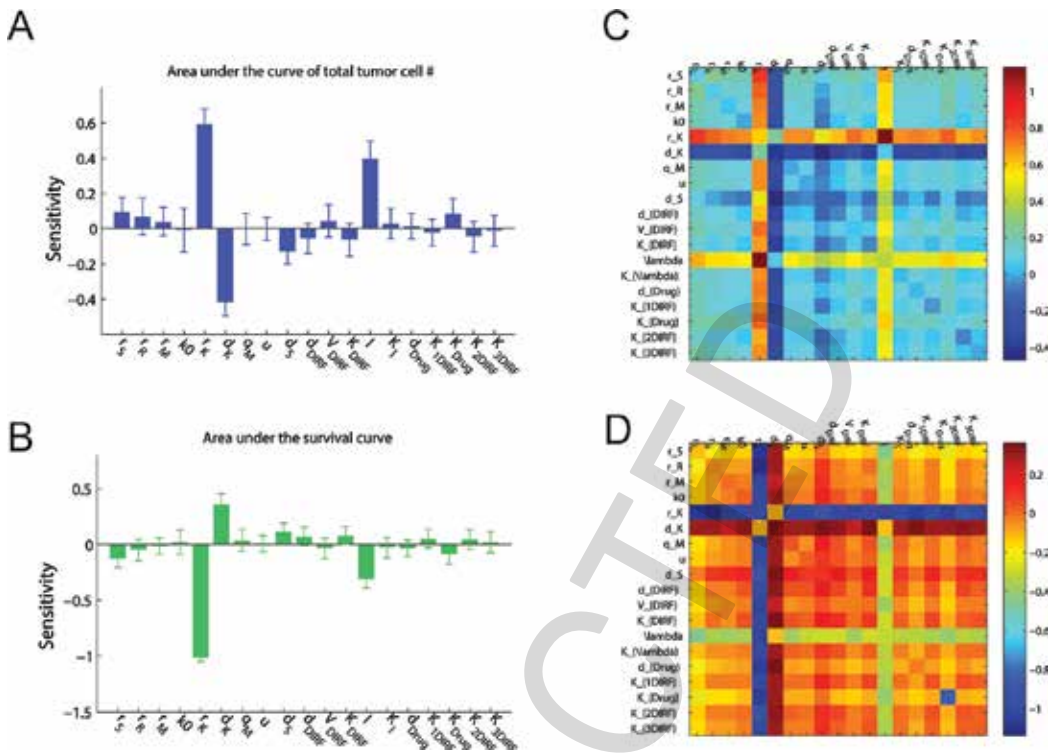

Figure 8. Sensitivity analysis. (A, B) single-parameter sensitivity analysis. Sensitivity coefficients for (A) area under curve of total tumor cell number, and (B) area under the survival curve in response to variations in different parameter values. The computations were repeated 20 times, and the mean value and standard deviation of the sensitivity coefficients were calculated. The critical parameters involved in this model include r_K, d_K, and λ. (C, D) two-parameter sensitivity analysis. The relative changes of the areas under the curves of (C) the total tumor cell number and (D) patient survival percentage with respect to the combinatorial variations in parameter values.

analysis result revealed some interesting phenomena. For example, the combination of r_S (growth rate of sensitive cells) and r_K, the combination of d_S (drug-induced death rate of sensitive cells) and d_K, the combination of r_R (growth rate of resistant cells) and r_K, and the combination of r_K and λ show significant impacts on the tumor growth and patient survival. This also suggests that combining anti-angiogenic therapy with targeted therapy to combat drug resistance and cancer progression may improve cancer patient survival.

4. Discussion

In this study, to examine therapy-induced drug resistance and cancer metastasis, we designed a novel stochastic model that connects the biological mechanisms underlying cancer drug resistance to population-level patient survival. A set of stochastic differential equations (SDEs) was used to model the dynamics of heterogeneous cellular populations containing drug-sensitive,

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drug-resistant, and metastatic cancer cells. An associated random variable characterizing progression-free survival time was subsequently defined. Our approach revealed several interesting features associated with cancer metastasis and progression kinetics. For example, dose-dependent synergy was evident in both BRAF-I and MEK-I co-treatment and BRAF-I and PI3K-I co-treatment. This result suggests that combination therapy with optimized dosages of inhibitors might reduce drug resistance.

Our model demonstrated that cancer metastasis and progression occur in bursts (**Figure 3D, F** and **Figure 6A**). As such, metastasis may occur earlier than can be detected using common radiographic imaging approaches [59]. Furthermore, metastatic cancers may quickly progress after being detected. Based on these phenomena, new prognostic methods that offer much earlier detection of metastasis and progression are needed. The identification of biomarkers that can be easily and regularly measured in cancer patients to detect cancer metastasis would be promising. Recently, ctDNA [60] has been acknowledged as a promising biomarker for several types of cancer [52, 61–64]. Using new PCR technologies, ctDNA can be quantitatively measured in cancer patients [65, 66]. Dynamic changes in ctDNA levels might serve as a biomarker of early relapse in cases of surgically resected metastatic melanoma.

We also investigated the impact of heterogeneity in patient drug-metabolism rates on survival percentage. To include the effects of population heterogeneity in our mechanistic models and examine the impact of patient drug metabolism heterogeneity on survival percentage, we considered three patient subclasses with different metabolic rates (low, medium, and high). As shown in **Figure 9**, metabolic rate differences significantly affected the probability of patient survival. The patient subclass with a high rate of drug metabolism showed a lower survival probability compared to the patient subclass with a low rate of drug metabolism. This was true under both the 3 weeks on/1 week off treatment schedule (**Figure 9A**) and the daily treatment schedule (**Figure 9C**). In **Figure 9B, D**, the effects of different drug combination dosages on survival percentage in high-metabolism patients are shown. An appropriately elevated dosage of BRAF-I combined with MEK-I (2-fold of normal dosages) improved progression-free survival in cancer patients with high metabolic rates. As a strategy for personalized therapy, optimizing dosing based on patient metabolic rate might be beneficial. Furthermore, our model indicated that daily treatment of cancer patients (**Figure 9C, D**) resulted in higher survival rates versus the 3 weeks on/1 week off treatment schedule (**Figure 9A, B**). These results suggest that daily drug treatment might produce better results than therapies with drug discontinuance.

Our model predicted that combined BRAF and MEK inhibitions produced a different pattern of synergy from that created by combined BRAF and PI3K inhibition. As demonstrated in [15], the use of a BRAF-I can induce drug-sensitive cells to secrete DIRFs that promote PI3K/AKT/mTOR pathway activation in drug-resistant cells. Thus, BRAF-I and PI3K-I co-treatment represses the actions of DIRFs on drug-resistant cells and reduces growth, dissemination and metastasis in drug-resistant cells. Therefore, at appropriate dosages, this combination therapy produced a synergistic effect, reducing the number of tumor cells (**Figure 5C, D**). Conversely, because MEK is a downstream target of BRAF signaling, MEK inhibition does not necessarily strengthen BRAF inhibition. As such, BRAF-I and MEK-I co-treatment produced only weak

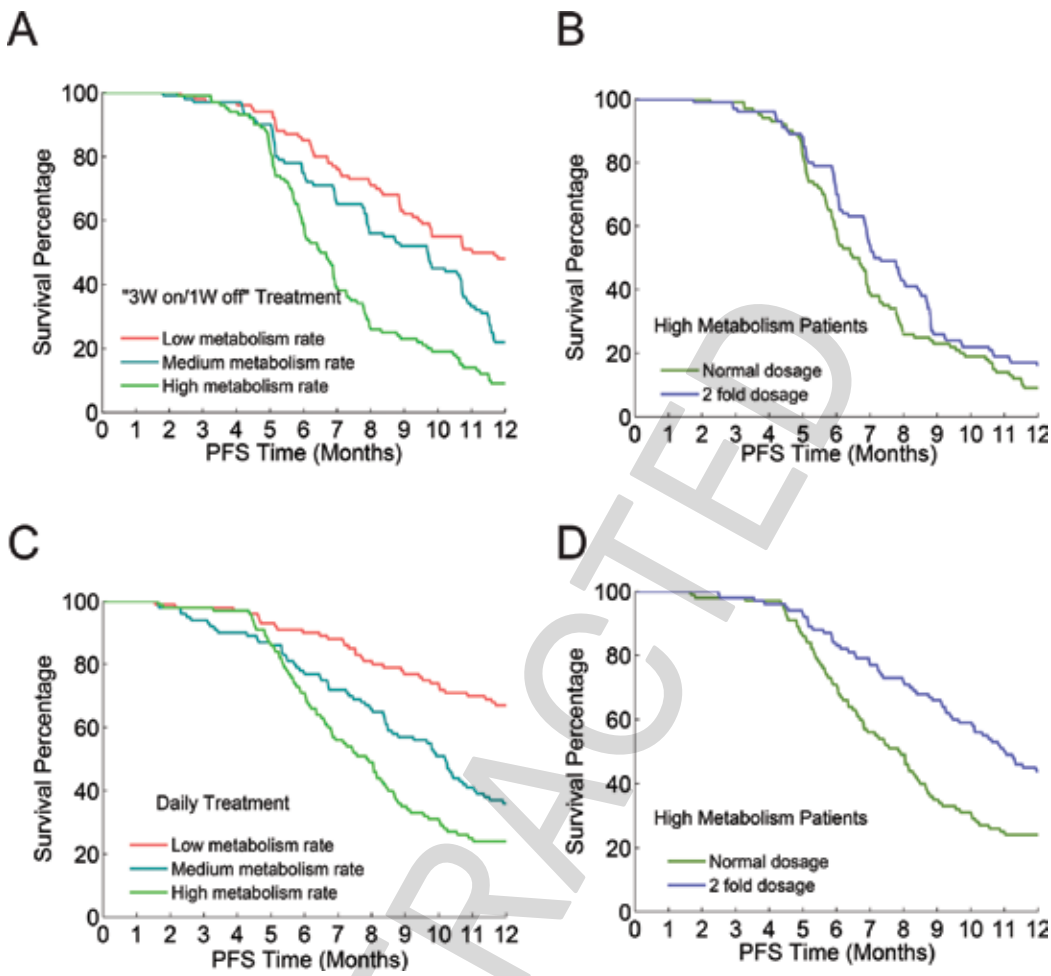

Figure 9. Impact of patient metabolic heterogeneity on patient survival. (A) Impact of heterogeneity in patient drug metabolism rates (low, medium and high) on survival percentage under a 3 weeks on/1 week off treatment schedule. (B) Survival percentage of patients with high metabolisms under a 3 weeks on/1 week off treatment schedule using different dosages of BRAF and MEK inhibitors (BRAF-I and MEK-I) in combination. (C) Impact of heterogeneity in drug metabolism rates (low, medium and high) on survival percentage under a daily treatment schedule. (D) Survival percentage of patients with high metabolisms under a daily treatment schedule using different dosages of BRAF-I and MEK-I in combination.

synergistic effects at relatively low doses. This lack of cooperation led to a smaller reduction in tumor cell number (**Figure 5A, B**).

Drug resistance is an obstacle often encountered during oncoprotein-targeted therapy and develops by very complex mechanisms. Tumors frequently display a substantial amount of spatial heterogeneity in both cell population composition and microenvironment factors, such as drug, oxygen and glucose concentrations [2, 67]. Recent studies have suggested that the emergence of drug resistance is driven by the tumor microenvironment [2]. In our study, based

on recent clinical and preclinical data, we modeled feedback in drug-sensitive cancer cells in response to drug treatment and interactions between heterogeneous cell populations and their microenvironments to understand drug resistance and metastasis dynamics in tumors. In future work, we aim to investigate spatial heterogeneity in tumor cells by developing an agent-based model [21, 43] using partial differential equations to assess spatial-temporal changes in the tumor microenvironment.

In summary, our study utilized a set of SDEs to model the dynamics of targeted therapy-induced drug resistance and metastasis. The model predicted that different patterns of synergy exist for the combination of BRAF-I and MEK-I and for the combination of BRAF-I and PI3K-I. Our study provides insight into the microenvironment-mediated mechanisms underlying drug resistance and delineates the implications associated with optimal dosing and scheduling of combination therapy for melanoma patients with BRAF mutations. It is our hope that this predictive model will facilitate cancer clinical trial design and accelerate the design of effective and robust tumor therapeutics.

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Chapter 4

Understanding the Anti-Tumor Properties Mediated by the Synthetic Peptide GK-1

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Additional information is available at the end of the chapter

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Abstract

Cancer exhibits adaptive features typical of complex systems, like resilience and robustness to environmental challenges through the emergent co-evolution of its components. These events promote carcinogenesis through dynamic interactions among numerous components and subsystems, including the immune system. During the past decade, our research group has provided substantial evidence that the peptide GK-1 has important immunomodulatory properties. In elderly mice, GK-1 acts as a potent adjuvant of the influenza vaccine through a mechanism that involves the activation of antigen-presenting cells (APCs) and an increased production of pro-inflammatory cytokines and chemokines (IFN-γ, TNFα, CCL2). To date, there is solid evidence supporting the antitumoral properties of GK-1 in murine cancer models. First, a lower occurrence and smaller size of spontaneous bronchiolar adenomas were found in elderly GK-1-treated mice compared to paired untreated mice. In two independent studies, GK-1 treatment reduced tumor growth and increased mouse survival in a murine model of melanoma and breast tumor. In the former model, a synergy between GK-1 and anti-PD-L1 treatment was observed, while in the latter, GK-1 alone controlled the metastatic burden. The effective activation of APCs induced by GK-1, restoring the antitumor-specific immunity, may underlie some of its antineoplastic effects.

Keywords: GK-1 peptide, melanoma, breast cancer, immunomodulator, antitumor

1. Introduction

1.1. Immunomodulation

Immunosurveillance comprises interactions between the immune system and cancer cells that take place even before the tumor formation [1, 2]. This process includes the recognition and control of transformed cells through antitumor immune responses, with three related outcomes: elimination, equilibrium, and escape [1–5]. In this regard, stimulating the innate immune system by immunogenic cells plays a role in the removal of incipient tumors, activating cells from the adaptive response like T and B cells, as well as promoting acute inflammation due to the concomitant production of immunostimulatory cytokines. Nevertheless, some transformed cells may not be eliminated. This escape phase of immunosurveillance is characterized by tumor growth promotion through a phenomenon called tumor-induced tolerance, which involves an increased expression of immunosuppressive components such as myeloid-derived suppressor cells (MDSC), regulatory T cells (Tregs), as well as T cell exhaustion and the production of immunosuppressive soluble factors [6, 7]. Indeed, some of these cells could be used as prognosis factors, since increased numbers of Treg and MDSC cells are related to a poorer outcome in cancer patients [8–13]; by contrast, a Th1 response is associated with a good prognosis in melanoma, breast, head, neck, colorectal, prostatic, and renal cancer [14–16].

The immune response can be modulated by compounds capable of enhancing (immunopotentiation) or diminishing (immunosuppression) the immune response, either in an antigen-specific or in a nonspecific manner; the latter implies that the immune system requires to be stimulated to restore the patient's immunocompetence. Immunomodulators are biological or nonbiological substances that can modify one or more components of the immunoregulatory network to achieve a specific antitumor immunity, such as inducing effector tumor-specific cytotoxic T lymphocytes (CTLs), activating macrophages and natural killer (NK) cells, and/or promoting the production of inflammation mediators [17–21].

Immunomodulators include adjuvants, vaccines, and immunoglobulins used to prevent or treat infectious diseases. They are characterized by their ability to activate cells of the innate immune system, mainly dendritic cells (DCs) and macrophages. Some examples of this type of agents are pathogen-associated molecular patterns (PAMPs) and molecules like squalene, aluminum salts, and peptides, which are often used as adjuvants in vaccines [21, 22].

1.2. Peptide-based therapies

Anticancer strategies based on peptides have several advantages over other chemotherapeutic approaches, like being nongenotoxic or possessing adjuvant properties; they also have a strong specificity, high affinity, good tissue penetration, and low toxicity with respect to small-molecule drugs and monoclonal antibodies [23–26]. Examples of anticancer peptides are (1) necrotic peptides (some of them are expressed in a wide diversity of species, including insects, fish, amphibians, and mammals, e.g., cecropins A and B found in mammals and various insects) [27]; (2) apoptotic peptides, cationic peptides known as host defense peptides (HDP) such as the bovine lactoferricin, magainin 2, hCAP109-135 (comprising the C-terminal

domain of human CAP18), and BMAP-28 from bovine myeloid cathelicidin [28–31]; (3) blocking peptides; (4) receptor-interacting peptides; (5) peptides that bind to cell-adhesion proteins; (6) protein kinase inhibitors; (7) protease inhibitors; (8) peptides with antiangiogenic properties; and (9) peptides with immunostimulatory activity [27].

With regard to receptor-interacting peptides, compounds like CpG, imiquimod, poly I:C (toll-like receptor (TLR) agonists), α-GalCer (glycolipid ligands), GM-CSF, IL-2, and IFNα/β have antitumoral activity, as well as adjuvant properties [32-34]. These compounds are capable of directly or indirectly enhancing APC functions and T effector activity. In this sense, some of the most employed immunotherapeutic agents in polytherapy induce the effector function of tumor microenvironment (TM)-associated T cells and macrophages [35–38]. For instance, CpG was a promising cancer immunotherapy adjuvant due to its capacity to induce a Th1 immune response and activate APCs through TLR9 signaling [35–38]; however, it failed to stimulate the immune response in clinical trials [39]. The identification of new adjuvants showing low toxicity and capable of stimulating a cellular Th1 response in humans would be a great advancement in the development of vaccines for infectious and noninfectious diseases such as cancer [40].

Unfortunately, several immunostimulators have failed to revert the immunosuppressive conditions in TM. For example, IL-2, IL-12, GM-CSF [41–44], and immunological adjuvants administered with highly immunogenic antigens like incomplete Freund's adjuvant, bacillus Calmette-Guerin [BCG], and MF59 have shown disappointing results [43, 44]. Moreover, these compounds have been associated with toxic effects [45–47].

2. Identification of the GK-1 peptide

Based on the nonspecific reactivity and immunopotentiator properties of GK-1, our group has been studying it as a promising adjuvant for cancer immunotherapy. This 18-amino acid peptide was first derived from the KETc7 protein, isolated from a *Taenia crassiceps* cysticercus cDNA library [48]; KETc7 is part of a broad family of proteins associated with membrane processes [49]. When searching for T cell epitopes *in silico*, GK-1 exhibited a strong association with MHC-I and, to a lesser extent, with MHC-II [49]. The immunomodulatory properties of GK-1 are associated with an efficient activation of cells involved in antigen presentation (such as DCs) by promoting the expression of the costimulatory molecules CD86 and MHC-II, as well as the secretion of soluble pro-inflammatory factors like IFN-γ, TNF-α, and CCL2 [50]. GK-1-treated DCs enhanced the proliferative response of antigen-specific CD4+ T cells both *in vivo* and *in vitro* [50]. GK-1 also induced the proliferation of CD8+ T cells and higher IFN-γ levels [51] even in the absence of adjuvant [52]. Considering that this peptide can promote APC function and enhance Th1 cell effector pathways, its capacity as an adjuvant of the influenza vaccine was evaluated. GK-1 increased the levels of specific IgG antibodies *in vivo,* before and after infection, in a murine model of influenza in elderly mice [53], favoring virus clearance after infection in both young and aged mice, which could be associated with an early infiltrate of mononuclear cells (lymphocytes and macrophages) to the lung parenchyma following the GK-1 peptide co-administration. Furthermore, lung histological examination showed better preserved alveolar spaces and less congested alveolar walls with respect to the vaccine-only animals [53].

3. GK-1 as an anticancer immunotherapy

In neoplasms, the host is often immunocompromised due to the presence of immunosuppressive cells and molecules in the TM, to prevent the removal of cancer cells [2]. This highlights the relevance of stimulating the host immune response against cancer antigens by administering immunoadjuvants along with chemotherapy, radiotherapy, or surgery [54]. In this regard, small peptides with a nonspecific immunostimulatory response like GK-1, long known to act as vaccine adjuvants, are potentially useful in cancer therapy. The antitumor effect of GK-1 has been studied in melanoma and breast cancer murine models.

3.1. GK-1 in a mouse melanoma model

Melanoma is the most malignant form of skin cancer, mainly affecting the Caucasian population [55, 56]. Until recently, systemic therapy for metastatic melanoma had been inefficient, with a 5-year survival rate for patients (<30%) [57, 58]. However, new therapies were recently approved to treat melanoma, such as pegylated-interferon-α2b (IFN-α2b) in the adjuvant setting; ipilimumab, an anti-CTLA4 monoclonal antibody, for metastatic disease; vemurafenib, an oral BRAF inhibitor indicated for patients with metastatic melanoma harboring BRAFV600 mutations, and more recently antibodies against PD-1 like pembrolizumab [59–61] and antibodies blocking PD-L1 pathways, as well as inhibitors of the mitogen-activated protein kinase (MAPK) pathway. Additionally, nonspecific immunomodulation by several cytokines (IL-2, IL-12, TNF-α, and IFN-γ) and TLR ligands [62–64] in addition to adoptive transfer approaches have been widely used [65]. For over a decade, DCs have also been used in immunotherapy against various types of cancer [66–68] as an alternative to chemotherapy, by vaccination with DCs loaded with tumor peptides (i.e., MAGE-AX [69–72] and/or with necrotic or apoptotic tumor cells to induce effector tumor-specific T cells [73, 74]). The efficacy of this immunotherapeutic approach was also evaluated against murine melanoma, using GK-1 as an immunostimulant.

GK-1 has been reported to increase the mean survival and significantly delays tumor growth in a melanoma model with B16-F10 cells, showing more necrotic areas along with the presence of numerous neutrophils (**Figure 1**). Neutrophilia inside pulmonary blood vessels was also observed, without evidence of macroscopic or microscopic metastasis. In a melanoma lung metastatic model, GK-1 decreased lymphocyte count, while increased the number of neutrophils and decreased the serum levels of IFN-γ; on the other hand, an increase in the levels of IFN-γ and IL-12 in the intratumor (lung metastases) environment, along with a decrease in IL-17, IL-4, IL-22, and IL-23 was also observed [75, 76]. The antitumor activities of IL-12 have been established in preclinical studies against various tumor cell lines; the increased concentration of the antitumor cytokine IL-12 found in primary tumors may enhance the damage to tumor cells, limiting the number of cancer cells detaching from the primary tumor [77–79]. Its antitumor activity is also mediated by the induction of IFN-γ [67, 78, 80], which upregulated the expression of MHC-I and -II by B16 cells *in vitro*, favoring a cytolytic response in MHC-I-restricted CTL (**Figure 1**) [81]. There is a consensus that the induction of a Th1 profile or the release of cytokines like IFN-γ and TNF-α by T cells is essential for an effective antitumor immune response in

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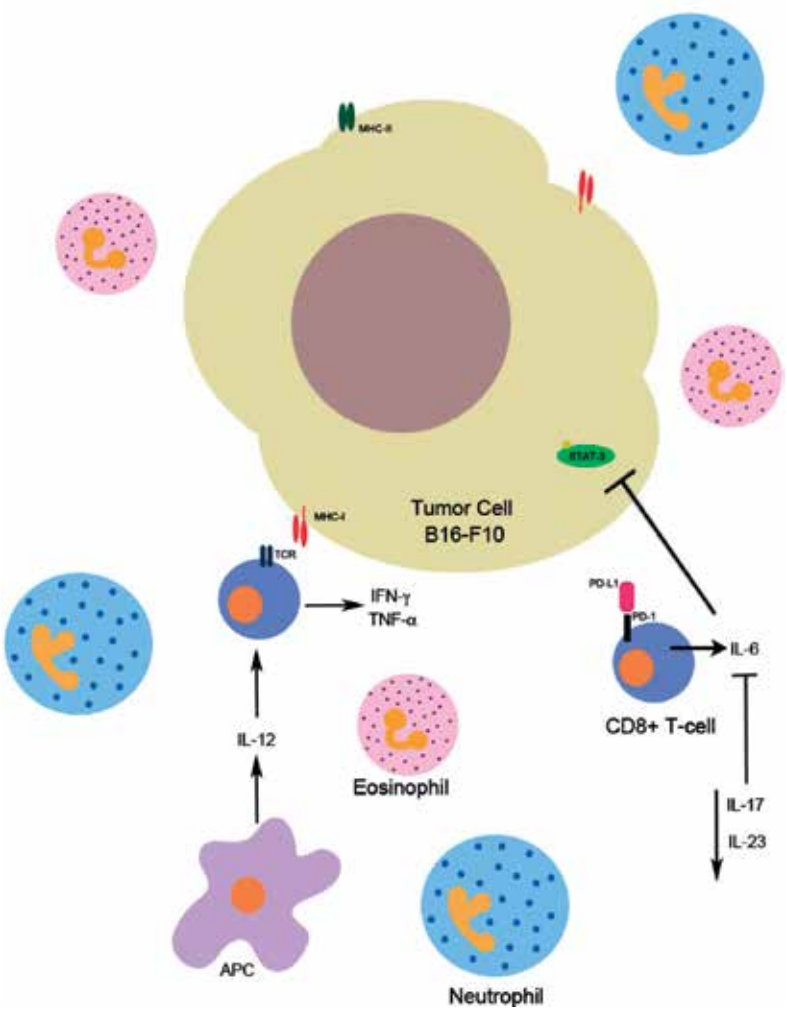

Figure 1. GK-1 in a preclinical mouse melanoma model. In a melanoma murine model with B16-F10 cells, GK-1 led to an increase in neutrophils with the increase of IFN-γ and IL-12 cytokines, along with a decrease in IL-17 and IL-23. On the other hand, the MAGE-AX/GK-1 treatment showed an increase in areas of cell death, characterized by eosinophilic regions and production of IFN-γ by CD8+ T-cells.

melanoma [82–84]. In fact, IFN-γ released from CTLs has been considered as a potent mediator of the antitumor response in bulky melanoma tumors [17, 85]. In contrast, IL-17 was proved to directly promote tumor growth and angiogenesis [86–88]. Indeed, it has been shown that IL-17 can promote tumor growth by a direct effect on IL-6 induction, which in turn activates STAT3 in both tumor and nontransformed cells in the TM [89]. Finally, IL-23 is an important molecular driver of Th17 cells in humans; IL-23 is increased in several tumors, and the expression of this cytokine antagonistically regulates local inflammatory responses in the TM, as well as the infiltration of epithelial lymphocytes [80]. Thus, the intratumoral subexpression of IL-17 and IL-23 in GK-1-treated mice could explain the reduced tumor progression (**Figure 1**).

Considering the capacity of GK-1 to enhance DC activation [50], BMDCs matured with TNF-α and stimulated with GK-1 and MAGE-AX were administrated to tumor-bearing mice in the melanoma model with B16-F10 cells; the treatment with MAGE-AX/GK-1 increased survival rates, while mice receiving GK-1 alone had a smaller increase in survival. Moreover, the combination MAGE-AX/GK-1 significantly delayed tumor growth and increased cell death areas, characterized by eosinophilic regions within melanomas. Similarly, both GK-1 alone and MAGE-AX/GK-1 increased the production of IFN-γ-producing CD8 cells, while GK-1 increased the percentage of IL-10 producing CD8+ T-cells [90]. The effect of MAGE-AX/GK-1 could be associated with higher levels of CD8+ lymphocytes in peritumoral lymph nodes, which have been correlated with the survival of patients suffering from melanoma and other cancer types [67, 91, 92].

Vera-Aguilera et al. [79] hypothesized that a combined GK1/anti-PD-L1 therapy could synergize and maximize the individual antitumor effect and extend survival. An increased survival was observed in mice treated with GK1/anti-PD-L1, as well as in mice treated with GK-1 or PD-L1 alone. Animals treated with GK1/anti-PD-L1 had smaller tumor masses. Additionally, GK1/anti-PD-L1 decreased the serum levels of IL-4, IL-5, IL-6, and IL-10. The mechanism by which the combined GK1/anti-PD-L1 treatment improved survival rates remains to be determined; however, the expression of PD-1 on T cells has been proved to be upregulated by IL-6 through the signal transducer and activator of transcription 3 (STAT3) [93], a point of convergence for several oncogenic signaling pathways leading to the expression of immunosuppressing molecules [94]. Similarly, the expression of PD-L1 and PD-L2 is also upregulated by numerous mechanisms, including the production of IL-4 and GM-CSF [93]. All these findings point to a possible synergistic mechanism associated with the reversion from an exhausted phenotype.

3.2. GK-1 in a breast cancer model

Considering the evidence described above, it is now clear that changes in the microenvironment could induce an antitumor response against the primary tumor and reduce the metastatic disease, which could allow us to control cancer progression. In this regard, immunomodulators like GK-1 can be used as anticancer therapies. In 2017, GK-1 was evaluated in a murine model of invasive breast adenocarcinoma, which spontaneously metastasizes to the lungs, liver, brain, and bone, similarly to breast cancer in humans [95–98]. GK-1 was associated with an increased survival in 4T1 tumor-bearing mice and a reduction in the primary tumor volume rate, which was accompanied by an increase of tumor cell death areas with morphologic features associated with necrosis (pyknosis, karyorrhexis, and karyolysis) and apoptosis (apoptotic bodies) at the primary tumors. These findings, along with an increase in IL-12 concentration in the primary tumor, denote deep changes in the TM induced by GK-1 [98], which could involve the infiltration of TCD8+, NK, and NKT cells in the primary tumor [77, 99, 100] (**Figure 2**). As described in the previous section, IL-12 has been associated with antitumor and antiangiogenic activities [100, 101], due to its capacity of inducing the infiltration of TCD8+ cells within tumor tissues [100]. In fact, it has been reported that a combined treatment with tamoxifen and IL-12 enhanced tumor inhibition due to an increase in apoptosis, and reduced tumor growth in a 4T1 cancer murine model [100].

Those changes suppose a TM that could reduce the tumor growth rate, and the concomitant reduction of cancer cell egress by detachment from the primary tumor, which allows them to invade the stroma and break the basement membrane. These changes could explain the reduction of pulmonary metastasis associated with the GK-1 treatment [98]. Additionally, changes in lung microenvironment associated with the GK-1 treatment have been reported. In this sense, a reduction in the concentration of b-FGF, CCL-3, GM-CSF, CCL-2, TNF-α, and CXCL-9, along with an increased concentration of IL-6 has been found [98] (**Figure 2**). These changes could reduce metastasis development, possibly by inhibiting the proliferation of cells that are essential for the growth of secondary tumors, such as macrophage-associated metastasis (MAM) and MDSC [102, 103]. Considering these results, GK-1 could change the tumor microenvironment, inducing an active antitumoral immune response that could lead to a decrease in cancer burden.

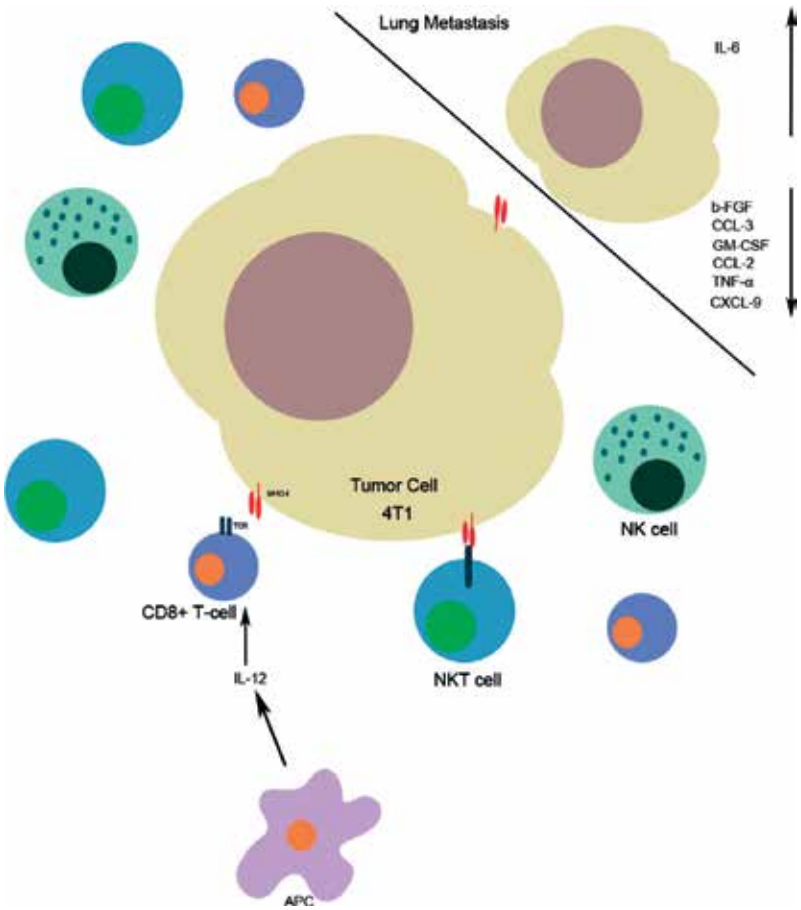

Figure 2. GK-1 in a breast cancer model. GK-1 was associated with an increased IL-12 concentration in the primary tumor, which could involve the infiltration of CD8+ T-cells, NK, and NKT cells. IL-12 is a cytokine produced principally by APC, such as monocytes, macrophages, and dendritic cells. This cytokine can induce specific CD8+ T-cells that are primed against tumor antigens and could serve as a tumor-specific CTL. Additionally, in the lungs, the GK-1-treatment induces a reduction in the concentration of b-FGF, CCL-3, GM-CSF, CCL-2, TNF-α, and CXCL-9, along with an increased concentration of IL-6, which correlates with a minor lung-metastatic burden.

4. Discussion

The ability of the GK-1 peptide to increase survival, significantly to delay tumor growth, and to reduce metastasis is discussed in this review. Considering that the immune system plays a crucial role in the outcome of cancer, orchestrating the response that may lead either to the control or dissemination of tumors [8, 78, 104], understanding the mechanisms that underlie the efficient response to the peptide is imperative.

It has been reported that the production of pro-inflammatory cytokines both by tumor and surrounding cells, along with the production of growth factors and chemokines, can promote the development of neoplasia by facilitating carcinogenesis programs, inducing a sustained cellular proliferative rate, inhibiting apoptosis and stimulating angiogenesis [105, 106]. As described above, GK-1 therapy contributed to decrease the levels of IL-4, IL-10, b-FGF, and GM-CSF; these chemoattractants, along with hypoxia, promote macrophage shift from a M1 to a M2 phenotype. M2-like tumor-associated macrophages (TAM) stimulate immunosuppression and increase blood vessel density, favoring angiogenesis. In a breast cancer model, lower CCL2 and CCL3 levels in the lungs of mice treated with GK-1 could be decreasing the migration of inflammatory monocytes such as MAM and MDSC, which promote metastasis [8, 13, 102, 107]. These changes in the microenvironment seem to contribute to control tumor burden and metastasis.

On the other hand, M1-like macrophages can contribute to tumor regression by recruiting cytotoxic CD8+ T (CTL) and NK cells [108–110]. In this regard, IL-12 induction by APCs could be contributing to the increase in the proliferation of CD8+ and CD4+ lymphocytes and the induction of a Th1 response, as previously reported [51, 52, 111]. Several studies have suggested a correlation of higher density levels of cytotoxic (CTL) and memory T lymphocytes (CD3+ CD45RO+) infiltrated in the primary tumor with increased survival rates of patients with different types of neoplasms [91, 112–116].

According to recent findings, the GK-1 peptide can induce a M1 phenotype and promote the efficient activation of DCs, which could be leading to the maintenance of an effector response against tumor growth, capable of counteracting the immunosuppressive response due to T cell exhaustion or DC dysfunction.

5. Conclusions

Considering the possible mechanisms of action of GK-1 and the information available, we propose that this peptide can decrease tumor growth and metastasis by changing the tumor microenvironment. GK-1 appears to reactivate the immune system affected by the tumor-associated suppressive microenvironment, thereby allowing immune cells to become activated. Although more studies focusing on the anticancer effect of GK-1 are required, this research gives new evidence on the possible clinical uses of GK-1 beyond its well-established adjuvant effect.

These results have also provided us with the rationale to evaluate the effectiveness of the GK-1 immunotherapy to revert the exhaustion of peripheral T-cells in several types of cancer.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

Abbreviations

APC	antigen-presenting cell
CCL-22	chemokine ligand-22
CTL	cytotoxic T lymphocytes
CTLA-4	cytotoxic T-lymphocyte antigen 4
DC	dendritic cell
HDP	host defense peptides
LAG-3	lymphocyte-activation gene 3
MDSC	myeloid-derived suppressor cells
NK	natural killer cells
PAMPs	pathogen-associated molecular patterns
PD-1	programmed cell death 1
PD-L1	programmed death-ligand 1
Treg	regulatory T cell
STAT3	signal transducer and activator of transcription 3
TM	tumor microenvironment
VEGF	vascular endothelial growth factor
VEGFR-2	vascular endothelial growth factor receptor 2

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Chapter 5

Sentinel Node for Accurate Diagnosis of the Head and Neck Carcinoma

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Additional information is available at the end of the chapter

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Abstract

When it comes to tumors of the head and neck, there is currently no reliable method for finding all the metastases of the neck. Therefore, we follow the rule of performing an elective dissection in the patients where more than 20% of metastases are expected. Even then, there are some patients with local recurrences. The explanation most likely lies in the incorrect histopathological diagnosis and unrecognized metastases. The ability to recognize smaller metastases can be accomplished by the use of the concept of the sentinel lymph node. This chapter describes the assessment of the neck status in 40 patients. In 18 patients, we have found metastases in the sentinel lymph nodes. It is important to note that in eight patients, metastases were found only after the use of serial cuts and immunohistological staining.

Keywords: sentinel node/s, serial section, selective neck dissection, head and neck cancer

1. Introduction

Metastases of the regional lymph nodes of the neck are the most important independent prognostic factor in the cancer of upper airways and upper digestive tract [1]. In the tumors of the same size, the presence of metastases of even one lymph node reduces the 5-year survival rate by 50% [2]. Despite the novel diagnostic methods, an accurate diagnosis of clinically nonsuspicious lymph nodes remains a big problem. There is currently no available method with which we could accurately find all the metastases of the regional lymph nodes. There are still a number of unsolved questions in regards to patients with a clinically negative neck. Thus, there is a dilemma of which investigative method gives us the most accurate results, do we need to treat a clinically negative neck, what is the optimal treatment, do we

need to treat the contralateral side of the neck, what are the chances of successful treatment of regional recurrences, and can we adjust the treatment to the etiology of the tumor [3].

Clinical examination with palpation is absolutely not sufficient for discovering small and occult metastases [4]. Newer methods such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) allow for a discovery of more occult metastases, but their use also results in a large number of false positive metastases [5]. With the use of CT and MRI, there is also a limitation of discovering the metastases of lymph nodes smaller than 5 mm [3]. The best results are achieved with the ultrasound investigation of the neck combined with a cytologic punction [6]. Currently, no method is able to identify all of the occult metastases [7].

Therefore, we perform an elective dissection in the patients where more than 20% of occult metastases are expected [8–10]. Despite this, there remain a certain percentage of patients in whom the regional recurrences of the operated neck occur [11, 12]. Why is that? How can we overcome this? The most likely explanation is the unrecognized metastases in the dissected lymph nodes of the neck, overlooked by the classical histopathological examination. This oversight leads to inadequate treatment (no adjuvant radiotherapy) in these patients. An accurate neck status can be acquired by the use of the concept of the sentinel lymph node.

There is a large increase of patients with changed classification even with the use of only a classical histopathological examination. According to the literature, the classification increases in 49% and decreases in 21% [13]. With the use of classical histopathological examination, the pathologist examines the removed lymph nodes with one or two cuts along the longitudinal axis, which can lead to the oversight of the smaller metastases. With the use of the concept of sentinel lymph node, the lymph nodes are examined with serial cuts and immunochemical staining. This means that we can discover all of the metastases and can accurately determine the classification of the neck, which leads to the discovery of additional patients needing the adjuvant therapy. If elective dissection and checking for metastases of the lymph nodes are not performed, and we delay the adjuvant treatment until the possible appearance of metastases of the regional lymph nodes, the prognosis is worse [14].

2. The concept of the sentinel lymph node

The sentinel lymph node is the one which drains the lymph from the tumor first [15]. We assume that the tumor thrombus stops in the first lymph node that it reaches, and if there are no metastases in this lymph node, there will also be none in the other lymph nodes.

This concept is widely accepted in the surgical treatment of the malignant melanoma of the skin, as well as in the surgical treatment of breast cancer [16, 17]. In the treatment of the malignancies of the head and neck, there is a problem, which was highlighted by O'Brien [18], in the use of the concept of the sentinel lymph node due to the proximity of the tumor and the sentinel lymph node, rapid spread of the radiocolloid in the lymphatic pathways, a large number of the lymph nodes accumulating the radiocolloid, and often small, hard to reach lymph nodes.

Even though these difficulties referred to the skin melanomas, we encounter the same problems in the surgical treatment of squamous cell carcinoma of the upper airways and upper digestive tract. This is one of the main reasons why the use of the concept of the sentinel lymph node in the head and neck tumors is not widespread. The other important reason is that we do not have a fast and accurate method with which we could intraoperatively discover all of the metastases of the sentinel lymph nodes [19, 20]. A big problem arises if a pathologist discovers after the surgery the metastases in the lymph nodes that were not discovered with a frozen section procedure. In these cases, a reoperation is required, usually done 3–4 weeks after the initial surgery. Although we know that this can happen, it is still a very unpleasant experience for both the patient and the surgeon.

The examination of the sentinel lymph node with serial cuts and immunohistochemical staining allows for the discovery of even the smallest metastases [21]. It is important that if no metastases are found in the sentinel lymph node, there were also none in the other lymph nodes [22].

3. Selective dissection of the neck with the identification of the sentinel lymph node

In the cases where more than 20% of occult metastases are expected, we decide for the selective dissection, which means the removal of the lymphatics of the regions in which the metastases are expected [8–10]. In the oral cavity cancer and the oral pharynx, this depends on the localization of the tumor. In the tumors that are close to the midline, we must make a decision about the unilateral or bilateral removal of the lymph nodes. The decision can be made easier with the use of scintigraphy which can show us where the lymph from the region of the tumor drains. We remove the region of the neck which contains the sentinel lymph and additionally two adjoining regions. If the lymph is draining to both sides of the neck, we perform a bilateral selective dissection.

It has been proven that the survival of the patients in whom the elective dissection has been done is increased compared to the patients where the dissection was only done after the appearance of the metastases [14].

The patient disfiguring after an elective selective dissection is relatively minor when performed by an experienced surgeon. However, there is always a certain esthetic deformation larger than the one where only a sentinel lymph node is removed. There is also always a risk of damaging the marginal, accessory, lingual, and hypoglossal nerves. This nerve damage is rare in a careful surgical dissection.

After the removal of the lymphatics of the determined regions, we identify and remove the sentinel lymph node from the dissected material. The node is then examined by the pathologist with the serial slices at 100–150 microns and immunohistochemical staining. The remaining lymph nodes are examined with the classical histopathological methods with one or two slices along the longitudinal axis and staining with hematoxylin and eosin.

4. Preoperative identification of the sentinel lymph node

Preoperatively we determined the neck status of all the patients with the ultrasound. The study included the patients with T1 and T2 cancers of the oral cavity and oropharynx in whom no suspicious lymph nodes were found by ultrasound.

Approximately, an hour before the surgical procedure, a lymphoscintigraphy of the tumor region was performed. The static and dynamic scintigraphy allowed for the localization of the sentinel lymph node, which was marked on the skin.

5. Scintigraphy

For the determination of the sentinel lymph node, we used the Tc-nanocolloid. We used Nanocoll, produced by Amersham Health (Italy). Nanocoll is a set for the preparation of 99 m Tc-albumin nanocolloid. At least 95% of the parts of this colloid are equal or smaller than 80 nm.

We used 2–4 mCi, which correlates to 7,4–14,8 mBq. The amount of the radiocolloid used depended on the size of the tumor. Radiocolloid was injected at four different areas in close proximity to the tumor (above, below, left, and right; or clockwise—12, 3, 6, and 9 o'clock). By changing the needle positioning, we are able to infiltrate by colloid the entire tumor region. After the injection of the radiocolloid, the patient rinsed their mouth with water to remove any possible radiocolloid residues which might have influenced the investigation. 10 minutes after the application of the radiocolloid, we started to follow its movement along the lymphatics into the closest lymph nodes. For the detection of the radiocolloid, we used a gamma-ray camera Picker SX 300. Dynamic scintigraphy lasted between 45 and 60 min.

After an hour, we also made static recordings in the anteroposterior and lateral projections. Static scintigraphy allowed for the marking of the sentinel lymph node location on the overlying skin of the neck. If we were unable to identify the sentinel lymph node after an hour, we repeated the static recordings again in 30 min.

In this way, with the use of static and dynamic scintigraphy, we were able to identify the correct sentinel lymph nodes and limit any possible "skip" metastases [23].

6. Intraoperative identification of the sentinel lymph node

After we localized the sentinel lymph nodes with scintigraphy, we performed an elective selective dissection. After raising the subplatysmal skin flap, we injected the methylene blue dye to the same area where scintigraphy was performed before.

For the methylene blue dye, we used a Patent Blue V dye (Laboratorie Guerbet, Aulanay-Sous-Bois, France). The amount injected depended on the size of the tumor (from 0.5 to 2 ml) since the entire area of the tumor had to be filled. We observed the spreading of the dye and

identified the sentinel lymph node as the one which colored blue. With the use of gamma-ray detector, we confirmed the accumulation of the radioisotope in the lymph node.

If the accumulation was 3-times larger than that of its surroundings, we considered it as a warm node, and therefore as our sentinel lymph node. Most of the sentinel lymph nodes turned blue and accumulated the radioisotope. However, there were some lymph nodes which only turned blue or only accumulated the radiocolloid, but not both. We treated these as the sentinel ones as well. We removed all the nodes that were assumed as the sentinel ones from the dissected material and sent them separately to a histopathological examination with the serial slicing. The rest of the lymph nodes were examined with the classic histopathological methods.

7. Examination of the sentinel lymph node and dissected material of the neck

The sentinel lymph node was prepared by the pathologist in paraffin blocks with serial cuts at 100–150 microns. With serial cuts, the alternating staining with hematoxylin and eosin and immunocytochemical staining with cytokeratin were used.

As a reagent for immunohistochemical staining, a reagent produced by Dako-Glostrup (monoclonal mouse anti-human cytokeratin clone AE 1/AE 3) was used.

All the remaining removed lymph nodes were examined with the classical methods of staining with hematoxylin and eosin in paraffin blocks with 1–3 slices along the longitudinal axis of the lymph node.

8. Results

Forty patients were treated with this method. In 18 out of 40 patients, we found metastases. In 10 of these 18 patients, the metastases were already discovered with the classical method (i.e., first cut of the sentinel lymph node). In 8 of the 18 patients with metastases, the metastases were only discovered after the serial slicing and immunocytochemical staining. In 3 of 10 patients in whom the metastases were already found with the classical method, the metastases were also found in the other lymph nodes, which meant that the N classification in these three patients changed from N0 to N2b. With the use of serial cuts of all sentinel lymph nodes, the classification changed from N0 to N2b in 4 of 7 patients.

9. Discussion

A final and accurate status of the neck provides us with a very good evaluation of the actual state of the neck and the need for eventual inclusion of the additional adjuvant radiotherapy. With the use of classical histopathological examination of removed lymph nodes after elective

dissection, we would have missed 8 out of 40 patients who did have metastases. Even with the use of only histopathological examination of the removed lymph nodes, we discovered 10 patients with metastases of the regional lymph nodes. With the use of classical method, the occult metastases were therefore discovered in 25% of the patients. This is an expected proportion of patients with the occult metastases [24–26]. To these patients, we have to add the patients in whom we have found the metastases with the serial cuts of the sentinel lymph nodes. There were eight of these patients. We see that the number of patients with occult metastases was close to 50%. With the use of classical histopathological examination, we would have discovered only around a half of the patients with the occult metastases. We cannot afford to examine all of the removed lymph nodes by serial cuts and immunohistochemical staining, due to the lack of personnel and funding. In selective dissection of the neck, we remove on average 13–15 lymph nodes, which would amount to more than 200 slices needing pathologist examination. Still, the question whether we would discover more patients with occult metastases remains. If we accept the concept of the sentinel lymph node [27, 28] that if there are no metastases in the sentinel lymph node that there are also no metastases in the other lymph nodes, the only question remains if the correct sentinel lymph node was selected. With the use of radiocolloid and concurrent intraoperative use of methylene blue, the identification is very reliable [29]. Even with the surgical method, when we remove only the sentinel lymph nodes, we discover many occult metastases with serial slices [30] that we otherwise would not have. This method has some weaknesses, mainly the possibility of missed metastases intraoperatively which are discovered afterwards [31] leading to an additional surgical procedure. Another potential problem is that we do not remove the true sentinel lymph node, which can happen due to a limited access and consequent inability to visualize the lymph flow by methylene blue. We may only see if the lymph node has stained or not, but we may not see if any other lymph node before has stained as well. In selective dissection where to gain access, we raise the entire subplatysmal skin flap, and we have a good visualization of the lymph drainage from the area surrounding the tumor. The visualization is also good when we use the gamma-ray detector to identify the lymph nodes which we marked on the skin when performing the scintigraphy. We often see many smaller lymph nodes on a very narrow area and if we would rely on the gamma-ray detector only, we could easily misidentify the sentinel lymph node. We can observe that after the removal of the sentinel lymph node, only the patients with regional recurrent disease have a very poor prognosis [32]. The possibility for recurrence is greatly increased if we have a larger number of metastatic sentinel lymph nodes. Micrometastases and isolated tumor cells do not have a larger influence on the possible recurrence [32]. The reason for this could be an overlooked true sentinel lymph node and the possible changes of lymph drainage and metastases found at unpredictable locations later on. With selective dissection where we remove the lymph nodes from the region of the sentinel lymph node's location and two adjoining regions, there is basically no chance to not remove all the lymph nodes with metastases, especially if adequate ultrasound or CT diagnostics were performed preoperatively.

Another question is if all of the metastases of the removed lymph nodes are found. If with serial slices and immunohistochemical staining in the sentinel lymph node we discover the metastases, there is also a possibility that the metastases are present in other lymph nodes. In 7 out of 18 patients, these metastases were discovered, even with the classical examination only. Therefore, there is a small chance that we have not discovered all of the micrometastases and

isolated tumor cells in the lymph nodes. If we assume that all of the lymph nodes containing these have been removed, we can conclude that they are not necessary for the further disease course. Micrometastases supposedly do not have an influence on the possibility of regional recurrences [32].

10. Conclusion

We can see that we do not have a method to preoperatively prove smaller metastases of the lymph nodes of the patients with the cancer of the upper airways and upper digestive tract. Even after a selective dissection with classical histopathological methods of removed lymph nodes, around 10% of the patients with metastases are missed. With a somewhat modified use of the concept of the sentinel lymph node, we can significantly decrease this number by discovering basically all of the metastases of the regional lymph nodes. A prospective study is needed to prove if the smaller metastases have an influence on the patient's survival and if they should be treated with additional adjuvant radiotherapy. However, there is an ethical question of not providing additional therapy to the patients with smaller metastases in numerous lymph nodes, despite the fact that they would not be receiving it with the classical examination of the removed lymph nodes anyway. There are also regional recurrences in the patients who have had a selective dissection, and the pathologist did not find the metastases in the removed lymph nodes. The percentage of these recurrences indicates that this might be these unrecognized metastases.

Conflict of interest

This study did not receive any external funding and was conducted during standard patient treatment. Therefore, there are no conflicts of interest.

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Chapter 6

Thyroidectomy without Ligatures in Differentiated Thyroid Cancer

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Abstract

Technical improvements in thyroid surgery are nearly close with the progress of the vessels sealing systems. In all cases, we need to obtain a radical and safe thyroid excision. This chapter is conducted to evaluate the technical key point and the postoperative benefits of our procedure using vessels sealing devices in differentiated thyroid cancers. A prospective study, carried out in First Surgical Clinic, Emergency County Clinical Hospital Tirgu Mureș, Romania from January 01, 2013 to March 01, 2018, based on 100 consecutive patients, divided into two groups: first group without ligatures, using Small Jaw LigaSure™, and the second group operated by conventional procedure. Statistical analysis of some parameters (the thyroid pathology, operative time, hospitalization days, analgesic drugs, immediate postoperative complications and histopathological findings) shows that this procedure provides a total and "complete" removal of the thyroid specimen, with a decreased operative time and fewer hospitalization days.

Keywords: thyroid cancer, LigaSure™ small jaw, vessel sealing device

1. Introduction

Thyroid cancer represents approximately 3.8% of all new cancer cases having different genetics, histogenesis and evolution, requiring specific management protocols and consensus [1]. Although a diagnosis of cancer is frightening, the vast majority of thyroid cancers is highly treatable and in most cases curable with a multidisciplinary treatment in which surgery plays an important role.

In this chapter, we present the technical key points and postoperative benefits of the procedure, using LigaSure™ small jaw in differentiated thyroid cancer.

2. Short history of thyroidectomy

During the twelfth and thirteenth century, there were many speculations regarding the role and the function of the thyroid gland and surgeries have been done according to them [2]. Roger Frugardi was first, in 1170, to describe the extirpation of the gland using setons, hot irons and caustic powder [3].

Evolution of thyroidectomy is related to the advances of the technology even though in the nineteenth century this procedure was considered "barbaric horrid butchery" (by S. Gross) [4]. Later on, in 1880, Jules Boeckel of Strasbourg introduced the collar incision to thyroid surgery, and this approach was popularized, later on, by Theodor Kocher.

The thyroidectomy (near or total), as we know it today, began in the 1860s with the help of Billroth [5]. Thyroid surgery was undertaken before the physiology was understood leading to complications, including massive hemorrhage, infection or injuries of the surrounding structures, which were associated with morbidity and mortality rates of about 40% [4].

In 1880, Sandstrom discovered the parathyroid glands but the fact that hypocalcemia was the definitive cause of tetany was not accepted until the twentieth century [6].

Later, in the nineteenth and the beginning of the twentieth centuries, Theodor Kocher practiced a meticulous thyroidectomy being able to report a mortality rate of 1%. He also described the "cachexia strumipriva" in patients following total thyroidectomy. For his contribution to thyroid pathology, Kocher received the Nobel Prize in 1909 [7, 8].

In 1920, the advances in thyroidectomy reached the peak making Halsted to refer to this surgery as a "feat which today can be accomplished by any competent operator without danger of mishap" [1].

Nowadays, thyroid surgery can be performed with a low mortality as well as with low morbidity. In order to obtain such results, surgeon must be aware of the pathophysiology of the thyroid disorders and must know very well the cervical anatomy.

3. Differentiated thyroid cancer

According to the American Cancer Society, differentiated thyroid cancer (DTC) derives from thyrocytes and expresses the sodium iodine symporter. DTC includes papillary, follicular and Hurthle cell cancer and represents most of thyroid cancer (90%).

3.1. Papillary cancer

About 80% of the patients diagnosed with thyroid cancers present the form of papillary carcinoma. This form of papillary cancer develops slowly, and it usually affects only one thyroid

Papillary thyroid cancer	Variants
	Classic (usual)
	Clear cell
	Columnar cell
	Cribriform-morular
	Diffuse sclerosing
	Follicular
	Macrofollicular
	Microcarcinoma (occult, latent, small, microtumor)
	Solid
	Tall cell
	Warthin-like

Table 1. Variants of papillary thyroid cancer.

lobe. With a slow growth, the papillary cancers often spread to the locally lymph nodes. Even it also affects the local lymph nodes, this cancer responds well to the treatment and is rarely fatal. There are several subtypes of papillary cancers, more than 10 histological variants which are documented and can be seen in **Table 1** [9, 10].

3.2. Follicular cancer

Unlike the papillary form, the follicular cancer is the second most common thyroid cancer, affecting 10% of the persons diagnosed with thyroid cancer. It is more common in patients whose diet is poor in iodine. This type of cancer is characterized by the development of distant metastases, affecting organs such as the lungs and bones. The prognosis of this type of cancer varies depending on the degree of invasiveness. In the traditional classification of follicular thyroid cancer (FTC), there are two groups: minimally invasive and widely invasive [11–13].

3.3. Hurthle cell cancer

This type is also known as oxyphil cell carcinoma. About 3% of thyroid cancers are of this type. Most of the authors consider it as a form of follicular cancer [14].

4. Risk classification of DTC

The risk classification helps to predict the risk of local recurrence and developing metastases and the mortality in patients with DTC. It uses multiple staging systems which are based on a combination of the size of the primary tumor, specific histology, extrathyroidal spread of the tumor and the age at diagnosis.

The staging system most often used for thyroid cancer is the **TNM** (**Table 2**) system, which is based on three key pieces of information:

- T = the size of the tumor
- N = the spread to the nearby lymph nodes
- M = the spread to distant sites (metastasis)

The most common risk classification is based on the tumor node metastasis (TNM) classification:

- very low-risk group: pT1a, cN0/pN0, cM02;
- low-risk group: pT1b, pT2, cN0/pN0, cM0;
- high-risk group: pT3, pT4, each N1, all M1.

TX: Primary tumor cannot be evaluated
T0: There is no evidence of primary tumor
T1: Tumor limited to the thyroid, whose maximum dimension does not exceed 2 cm
T1a: Tumor limited to the thyroid, whose greatest dimension does not exceed 1 cm
T1b: Tumor limited to the thyroid gland with dimension >1 cm, but lower than 2 cm in greatest dimension
T2: Tumor >2 cm but ≤4 cm in greatest dimension limited to the thyroid
T3: Tumor limited to the thyroid or with minimal extrathyroidal extension, with dimension <4 cm
T3a: Tumor dimension >4 cm but is limited to the thyroid
T3b: Minimal extrathyroidal extension invading the strap muscles (sternohyoid, sternothyroid, thyrohyoid or omohyoid muscles) from a tumor of any size
T4: Includes extrathyroidal extension into neck major structures
T4a: Tumor of any size with extrathyroidal extension invading subcutaneous soft tissues, larynx, trachea, esophagus or recurrent laryngeal nerve
T4b: Tumor of any size with extrathyroidal extension invading prevertebral fascia or encasing carotid artery or mediastinal vessels
NX: Regional lymph nodes cannot be evaluated
N0: There is no evidence of regional lymph node metastasis
N1: Metastasis to regional nodes
N1a: Unilateral or bilateral disease involving level VI or VII lymph nodes metastasis (pretracheal, paratracheal, prelaryngeal / Delphian or upper mediastinal)
N1b: Unilateral, bilateral or contralateral lymph nodes metastasis (levels I, II, III, IV or V) or retropharyngeal lymph nodes
M0: No distant metastasis
M1: Distant metastasis

Table 2. TNM classification, 8th edition [15].

	Age at diagnosis <55 years		
Stage I:	any T	any N	M0
Stage II:	any T	any N	M1
	Age at diagnosis ≥55 years		
Stage I:	T1	N0/NX	M0
	T2	N0/NX	M0
Stage II:	T1	N1	M0
	T2	N1	M0
	T3a/T3b	any N	M0
Stage III:	T4a	any N	M0
Stage IVA:	T4b	any N	M0
Stage IVB:	any T	any N	M1

Table 3. Stage grouping according to AJCC.

Based on the risk of structural disease recurrence, the American Thyroid Association defines in their current guideline the next classification [16]:

- high-risk group: gross extrathyroidal extension, incomplete tumor resection, distant metastases, or lymph node >3 cm;
- intermediate-risk group: aggressive histology, minor extrathyroidal extension, vascular invasion, or >5 involved lymph nodes (0.2–3 cm);
- low-risk group: intrathyroidal DTC, ≤5 lymph nodes micrometastases (<0.2 cm)

The American Joint Committee on Cancer (AJCC) uses the combination of TNM classification and an age of more than 55 years at diagnosis as risk factor (**Table 3**).

5. Surgery without ligatures

Total complete thyroidectomy is the procedure that can be defined as an extracapsular thyroidectomy in which we remove the entire gland without remnant tissue.

5.1. Surgical instruments

The tool kit consists of a scalpel, Pean forceps (1–2 pieces), anatomical and surgical forceps, scissors, Farabeuf or Kocher spacers, monopolar cautery and LigaSure™ small jaw.

5.2. Incision

A 5–6 cm Kocher incision is performed in a flexion fold at 2–2.5 cm of the sternal notch. The skin, the subcutaneous cellular tissue and the platysma muscle are cut, after which a superior

and a lower flap is made by dissection using LigaSure™ or electrocautery. The upper flap is suspended, exposing the white line (**Figures 1–3**).

5.3. How we do it

Penetration into the cleavage space by cutting the medial raf and releasing the anterior face of the anterior thyroide lobe. The thyroid lobe is mobilized with a thread (**Figure 4**).The dissection is continued laterally by sealing the middle thyroid vein with the LigaSure™ small

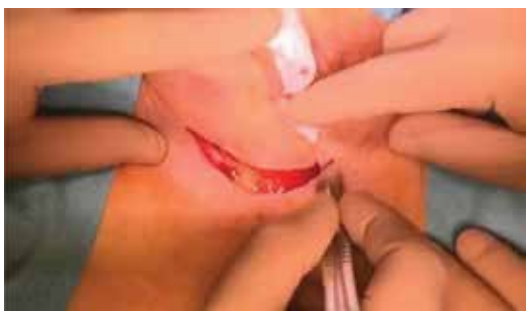

Figure 1. Kocher incision.

Figure 2. Preparing the upper and lower flap.

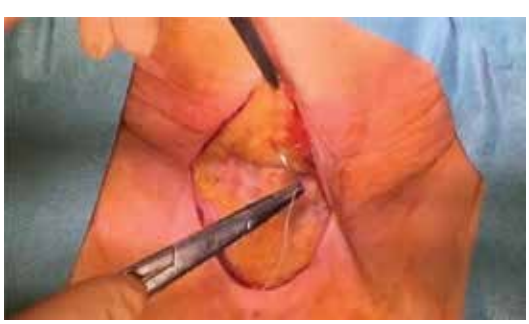

Figure 3. Suspending the upper flap.

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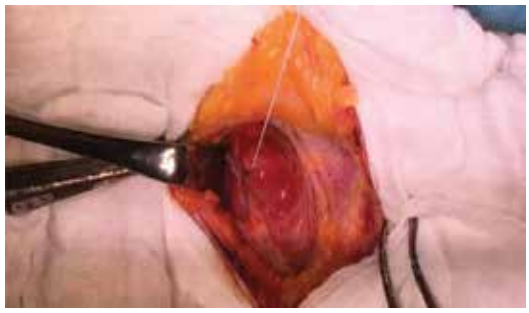

Figure 4. Traction of the thyroid lobe using thread.

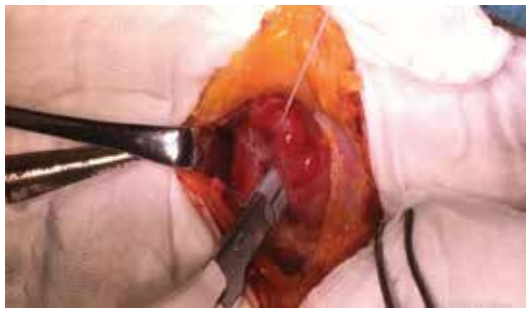

Figure 5. Lateral dissection with the sealing of the medial vein.

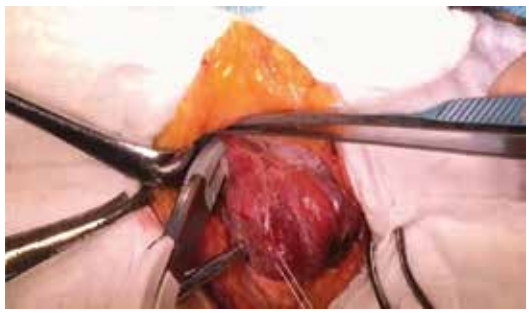

Figure 6. Sealing the upper thyroid pedicle.

jaw, then continuing cranially to the upper thyroid at the same time as the thyroid lobe dislocates (**Figure 5**).

Upper pole dissection with LigaSure™ *Small Jaw*, sealing the upper thyroid vascular pedicle (**Figure 6**).

The sealing of the lower branches of the lower thyroid pedicle is the essential time of this intervention, with the preservation of parathyroids, secondary branches of the inferior artery and the recurrent nerve. Dissection proceeds to the median line, sectioning Berry's ligaments.

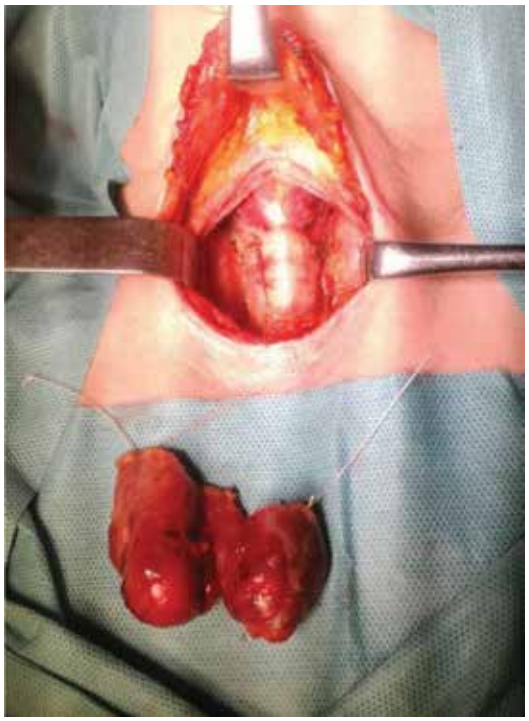

Figure 7. Monobloc resection of the thyroid gland.

Lobectomy ends with the separation of the isthmus, being an optional time, or we can achieve a monobloc resection of the gland (**Figure 7**).

Control of the hemostasis, drainage of the thyroid cavity, intradermal resorbable suture.

6. Evaluation of the technique

6.1. Study description

In order to evaluate the feasibility of the technique, we conducted a prospective study which was carried out in Clinic of Surgery I, Emergency Clinical County Hospital Târgu Mureș from January 01, 2013 to June 01, 2017. The study enrolled 100 consecutive patients divided into two lots: 50 who underwent total complete thyroidectomy using LigaSure™ small jaw, including 10 with "monobloc resection" and 50 in whom total thyroidectomy was performed by conventional procedure (by using ligatures). The two groups were compared using statistical analysis following the next parameters: the thyroid pathology, operative time, hospitalization days, analgesia used and immediate postoperative complications. Statistical analysis was performed employing Student's test for comparison of the continuous variables. Differences between nonparametrical variables were compared using the Mann-Whitney U-test. Descriptive data were reported as the mean ± SD. The level of significance was set at $p < 0.05$.

All patients have been prior diagnosed by fine needle aspiration (FNA) biopsy showing either papillary or follicular cancer.

All patients had preoperative and postoperative endocrinological consultations, and all the female patients had gynecological examination prior to the surgery.

6.2. Results

Extracapsular thyroidectomy was performed in all patients.

Surgery consisted of performing total thyroidectomy and double drainage of the thyroid compartment.

The two groups included in this study had similar demographics (age, sex) and the thyroid pathology also being the same (**Table 4**).

In the evaluation of the operative time measured from the moment of the skin incision to wound closure, we have found that statistical data followed a Gaussian distribution and it could be interpreted by Student's t-test. The mean operative time in group 1 was 73.81 ± 16.96 min, which is significantly less than the operative time in the second group (conventional) 106.19 ± 31.66 min (**Table 5**).

To appreciate the length of hospital stay, we applied the nonparametric Mann-Whitney test, since the two groups did not have a Gaussian distribution pattern, because of the value of 17 days in group 1 and of 19 days in group 2. For group 1, the mean hospitalization time was of 4 days (range: 2–17 days), and for group 2, it was also 4 days (range: 3–19 days) (**Table 6**). We have not found statistically significant differences relating to these parameters of the patients in the two groups, regardless of the statistic method applied.

	Group 1	**Group 2**
Mean age	54	52
Sex ratio F:M	45:5	43:7

Table 4. Patient's demographic characteristics.

Minimum	45	65
Mean	73.81	106.19
Maximum	120	200
Median	72.5	100
Standard deviation	16.96	31.66

Table 5. Distribution of patients depending to the operative time.

	Group 1	Group 2
Minimum	2	2
Mean	4.09	4.61
Maximum	17.00	19.00
Median	3.00	4.00
Standard deviation	2.95	3.21

Table 6. Distribution of patients depending on length of hospitalization.

	Group 1	Group 2
Low pitched voice	3	4
Haematoma	2	4

Table 7. Complications in the two groups.

The evaluation of postoperative analgesia was performed by measuring the dose of analgesic drugs administered to each patient. The nonparametric Mann-Whitney test had been applied considering that the two groups did not have a Gaussian distribution (because of a maximal value of 17 days observed in the LigaSure-group and a maximal value of 19 days identified in the conventional group). The mean period of analgesia in group 1 was 2.5 days (range 1–16 days), while in group 2, it was 3 days (range: 2–19 days). There was no statistically significant difference between the period of analgesic drug requirement in the two groups ($p = 0.06$).

No patients developed manifestations of hypocalcemia (hypoparathyroidism). There were few patients who experienced a change in voice (low pitched voice) seen in three patients from group 1 and in four patients in group 2. Hematoma was observed in two patients belonging to group 1 and in four patients belonging to group 2. There is no statistic significant difference between the incidence of early postoperative complications in the two groups (**Table 7**).

7. Discussion

Recently, thyroid surgery is considered quite safe, thanks to the proper surgical techniques that kept complications at a minimum rate (2–3%) [17].

The introduction of the new sealing devices in thyroid surgery benefits both patients and the surgeons. LigaSure™ small jaw is a proper device used to perform total complete thyroidectomy, and it gives some technical advantages like: creates a clean surgical field providing better visibility, observed in other study [18]; no need to expose the vascular pedicles their dissection being possible using the device's forceps; reduces the operative time, also observed by Gac, Cabane and Hou Shan Yao [19, 20]; performs safe sealing of blood vessels, observed

also by Marazzo and Lepner [20, 21]. This device can also remove the complications regarding the thread pathology.

The extracapsular total complete thyroidectomy can be performed without isthmectomy.

Dissection of vascular pedicles is relatively easy, allowing sufficient exposure to a very narrow anatomical space, and the separation and preservation of the integrity of the laryngeal nerves and parathyroid glands are possible in the absence of invasion by the pathological process (tumor or inflammatory).

By lower temperature dispersion (1–2 mm) from the device's jaws, the nerve elements are spared, detaching the thyroid by cutting the Berry ligaments and avoiding burns in the laryngeal tracheal conduit.

In order to avoid the possible thyroidectomy complications such as parathyroid trauma or laryngeal nerves injuries, a good hemostasis becomes the priority for the thyroid surgeons. Hemostasis achieved by classic methods such as tie and clamp, electrocautery, clips is time consuming and can lead to knot slipping and thermal trauma of the surrounding tissues [22].

Nowadays, minimally invasive surgical techniques are used on a large scale in other surgical fields but thyroid gland resections, for both benign and malignant tumors are rarely performed. Zorron et al. described an endoscopic approach in patients without preexisting neck operations using transoral-vestibular approach but with the limitation of the study due to the need of the evaluation of the technique [23].

8. Conclusion

Total and "complete" thyroidectomy represents a feasible technique in our days.

In our opinion, based on the results, this technique can be considered safe.

Using the vessel sealing devices brings real benefits both for patients and surgeons.

In surgical services that do not have assisted video surgery, this technique can be considered "gold standard" in selected cases.

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Conflict of interest

There is nothing to declare.

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Chapter 7

Quality of Life Following Intersphincteric Resections for Low Rectal Cancer: Early Results

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Claudiu Varlam Molnar and Vlad-Olimpiu Butiurca

Additional information is available at the end of the chapter

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Abstract

Intersphincteric resections are part of the therapeutic arsenal that preserves the sphincterian apparatus. This chapter analyzes the evolution of rectal surgery leading up to intersphincteric resections, deals with anatomical and oncological aspects in rectal cancer, and finally shows our own personal experience with ISR in a series of 40 cases focusing on oncological outcomes, continence, and defecation. As a conclusion, intersphincteric resection represents a feasible therapeutic option in highly selected cases that exempts the patient from the need of a permanent colostomy bag without compromising oncological principles. The Wexner score system is simple and effective in objectifying continence in patients that undergo this type of surgery.

Keywords: intersphincteric resection, ISR, TME, functional outcomes, oncological outcome

1. Introduction

1.1. History of rectal surgery

Surgery for rectal cancer has been around ever since 1907, when William Ernest Miles performed the first rectal surgery with radical intent known today as abdominoperineal resection (APR) [1]. The intervention devised by Miles quickly gained followers, and for years, it established itself as a gold standard for rectal cancer. Miles based his procedure on the assumption that rectal cancer spreads in a cylindrical manner both downward and upward and thus both abdominal- and perineal approaches are necessary to completely remove the tumor. Sigmoid

division and rectal dissection were started from above and then completed in the perineal approach with total sphincter removal [1, 2]. The Milles procedure, albeit being seemingly satisfactory at the time from an oncological point of view, had a 58% 1-year survival rate and numerous complications. Even at that distant time, an important question arose regarding what the best level of arterial ligation and division should be in radical rectal resection [1]. That question is still to be answered.

Following Miles APR, Henri Albert Hartmann devised the Hartmann's procedure in 1921 or the anterior resection of the rectum that consisted of the removal of the rectum with preservation of the distal third, internal and external sphincter and a left flank permanent colostomy [1]. This intervention was designed as an alternative to APR in the case of proximal rectal tumors. The main goal of the Hartmann's procedure was to reduce complications and mortality rates. As previously stated, the indications for this procedure where tumors of the proximal rectum still leaving low rectum and mid rectum tumors with no other surgical option then APR. Both procedures left patients with a permanent colostomy that had a major impact on quality of life. At that time, the "colostomy bag industry" was underdeveloped and bags had frequent odor and leakage problems, eventually leading to patients refusing surgery that entailed a permanent colostomy. This obvious downsize of APR and Hartmann's procedure led surgeons to develop techniques that allowed the preservation of the sphincter and normal defecation.

World events and WWII left the development of rectal surgery techniques in obscurity and it was only in 1948 when Claude F. Dixon presented the results of restorative anterior resection for proximal rectum and distal sigmoid tumors that rectal surgery took its first steps into sphincter saving techniques [3]. His data published in Annals of Surgery, showed a 5-year survival rate of 64% [3], as compared to Miles's procedure that had a 58% 1-year survival rate.

In 1951, Golligher, Dukes, and Bussey proved that local tumor spread in rectal cancer did not exceed 2 cm from tumor margins (cancer cells were discovered at >2 cm from the distal margin of tumor in only 2% of 1500 analyzed specimens); therefore, the authors considered that a 5-cm margin of clearance would have ensured a reasonable radicality [4]. These figures changed in the following years: first a safety margin of <5 cm and down to 2.5 cm was considered acceptable, then, in 1983, Nichols proved that a safety margin of 2 cm allows the same radicality of more extended resections with no change in overall survivals; finally, in most recent years, no differences in oncological outcomes were found with a safety margin of even <1 cm [5–7].

The work of Gollingher, Dukes, Bussey, and Nichols set the premises for the development of sphincter saving techniques for tumors of the mid and lower part of the rectum. Their studies allowed surgeons to apply surgical techniques that exempt the patient from the need of a permanent colostomy bag while maintaining an acceptable oncological outcome. The decrease of the safety margin from <5 to <1 cm in rectal cancer surgery nowadays also led to the more detailed study in the anatomy of defecation and of the structures involved in continence. This interest of rectal surgeons coupled with patient request to avoid a colostomy bag following surgery set the premises for true sphincter saving surgery for mid and especially low rectal cancer.

One of the most important technological breakthrough in rectal cancer surgery was the development of surgical staplers. The diffusion of stapler technology and its application in surgery started in 1972 thanks to Mark Mitchell Ravitch who introduced stapler suturing in the GI tract [8]. The development of surgical staplers started in the Moscow Scientific Research Institute for experimental Surgical Apparatus and Instruments in the ex-Soviet Union after WWII and then found its way to the United States of America thanks to Dr. Nikolai Amosov [9]. The first use of circular staplers in rectal surgery was reported by Fain in 1975 who stated that the device made low colorectal anastomosis easier with a leakage rate like that of hand-sewn anastomosis [10]. The stapler was further refined in the 1980 by Knight and Griffen who introduced the double stapling technique for low colorectal anastomosis [11].

From 1980 to 1986, coloanal anastomosis, intersphincteric rectal dissection, and colonic-pouch anal anastomosis were introduced by Parks, Lazorthes, and Parc, respectively, with the aim to preserve or improve sphincter function even in low rectal tumors [5, 7].

In 1994, Schiessel further brought into light the intersphincteric resection for low rectal cancer by publishing a series of 34 patients with low rectal cancer that underwent ISR with good postoperative results focusing on postoperative continence.

1.2. TME: total mesorectal excision

The concept of TME was introduced by Heald in 1982 and it represents a true milestone in modern rectal cancer surgery [12]. As opposed to the interventions described by Miles, Hartmann, and Dixon that used blunt dissection of the mid and distal rectum without taking into account the subtle differences in anatomy of the rectum, Heald theorized that rectal cancer is "more apt to spread initially along the field of active lymphatic and venous flow" and that the mesorectal fascia itself is "impenetrable only in the sense of being an avascular interface between viscus and soma." He named the said space as the *holy plane* (**Figure 1**).

Heald's principle was grounded on the knowledge that "the plane which surrounds the mesorectum is created by its separate embryological origin;" whereas, the whole rectum and mesorectum, which have the same embryological origin, "are one distinct lymphovascular entity" [13].

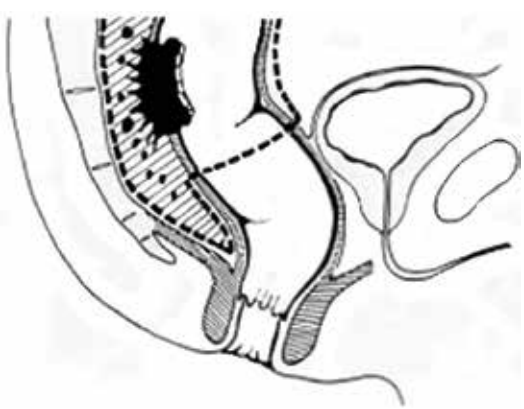

Figure 1. TME—Total mesorectal excision.

A surgical plane represents a "potential space between contiguous organs which can be reproducibly created by dissection" [13]. From a rectal surgeon's point of view, this space is indeed a *holy plane* and finding the right plane during rectal surgery can mean the difference between a radical intervention and a palliative one. Dissection along this plane should be done under visual control, be sharp and be done under gentle continuous traction [13].

Performing TME during rectal surgery for mid and low rectal tumors caused an increase in survival rate toping at 80% at 5 years following surgery and a local recurrence rate of only 4%, thus proving Heald's hypothesis [14].

1.3. Anatomy

The anal canal is the most terminal part of the lower gastrointestinal tract that lies between the anal verge (anus, anal orifice) in the perineum below and the rectum above.

The demarcation between the rectum above and the anal canal below is the anorectal ring or anorectal flexure, where the puborectalis muscle forms a sling around the posterior aspect of the anorectal junction, kinking it anteriorly. The anal canal is completely extraperitoneal. The length of the anal canal is about 4 cm (range, 3–5 cm), with two-thirds of this being above the pectinate line (also known as the dentate line) and one-third being below the pectinate line [15].

The pectinate line is the site of transition of the proctodeum below and the postallantoic gut above. It is a scalloped demarcation formed by the anal valves (transverse folds of mucosa) at the inferior-most ends of the anal columns. Anal glands open above the anal valves into the anal sinuses. The pectinate line is not seen on inspection in clinical practice, but under anesthesia the anal canal descends, and the pectinate line can be seen on slight retraction of the anal canal skin [15].

The anal canal just above the pectinate line for about 1–2 cm is called the anal pecten or transitional zone. Above this transitional zone, the anal canal is lined with columnar epithelium (which is insensitive to cutting). Anal columns (of Morgagni) are 6–10 longitudinal (vertical) mucosal folds in the upper part of the anal canal [15].

The anorectal junction or anorectal ring is situated about 5 cm from the anus. At the anorectal flexure or angle, the anorectal junction is pulled anterosuperiorly by the puborectal sling to continue below as the anal canal [15].

Levator ani and coccygeus muscles form the pelvic diaphragm. Lateral to the anal canal are the pyramidal ischioanal (ischiorectal) fossae (1 on either side), below the pelvic diaphragm and above the perianal skin. The paired ischioanal fossae communicate with each other behind the anal canal [15].

The anterior relations of the anal canal are, in males, the seminal vesicles, prostate, and urethra, and in females, the cervix and vagina with perineal body in between. In front of (anterior to) the anal canal is the rectovesical fascia (of Denonvilliers), and behind (posterior) is the presacral endopelvic fascia (of Waldeyer), under which lie a rich presacral plexus of veins. Posterior to the anal canal lie the tip of the coccyx (joined to it by the anococcygeal ligament) and lower sacrum [15].

The anal canal is surrounded by several perianal spaces: subcutaneous, submucosal, intersphincteric, ischioanal (rectal), and pelvirectal [15].

1.4. Blood supply

The anal canal above the pectinate line is supplied by the terminal branches of the superior rectal (hemorrhoidal) artery, which is the terminal branch of the inferior mesenteric artery. The middle rectal artery (a branch of the internal iliac artery) and the inferior rectal artery (a branch of the internal pudendal artery) supply the lower anal canal.

Beneath the anal canal skin (below the pectinate line) lies the external hemorrhoidal plexus of veins, which drains into systemic veins. Beneath the anal canal mucosa (above the pectinate line) lies the internal hemorrhoidal plexus of veins, which drains into the portal system of veins. The anal canal is, therefore, an important area of portosystemic venous connection (the other being the esophagogastric junction). Lymphatics from the anal canal drain into the superficial inguinal group of lymph nodes.

1.5. Nerves

The advent of laparoscopic surgery and the awareness of the importance of autonomic nerve identification and preservation during rectal surgery both had a positive impact on the patients' postoperative quality of life. The identification of pelvic splanchnic nerves, the bundle of Walsh, hypogastric nerves identifications and plexus saving techniques lowered the rate of postoperative sexual and bladder dysfunction according to Hojo et al. [16].

In the era of laparoscopic surgery, minimally invasive surgery and robotic surgery rectal nerves visualization and preservation have become significantly easier. This is due to the optical magnification by even more sophisticated camera systems including 3D imaging and to the intraperitoneal pressure that "shows the surgeon the correct plane."

Long-term results of the first randomized controlled trials (CLASICC RCT, COLOR II RCT, ACOSOG Z6051 Study), all done between 2013 and 2015, are available. The oncological results are questionable, but postoperative pain and quality of life resulted significantly better after laparoscopic rectal resections [17–19].

2. Material and method

2.1. Case series

We performed a prospective study on a group of 40 patients admitted in the First Surgical Clinic of the Tîrgu-Mureș Emergency County Hospital between 2015 and 2017. All patients were diagnosed with low rectal cancer. One of our main inclusion criteria was the flat denial of patients to have a permanent colostomy bag, even a temporary one. Patient's refusal was registered in the written informed consent. Tumor localization, tumor type, and the preoperative Wexner score were also considered alongside with preoperative antigen levels.

Most of the patients were from an urban background, 75% were males and none of them had a previous personal oncological background. Median age of the group was 66 years old.

2.2. ISR technique

Intersphincteric resections can be performed for type II (juxta-anal) or type III (intra-anal) low rectal tumors (<6 cm from the anal verge), cases in which partial intersphincteric resection is performed, respectively total intersphincteric resection [21].

The first part of the surgery starts with the primary vascular approach of the inferior mesenteric artery in all cases followed by left colon mobilization and ligation of the inferior mesenteric vein and TME (total mesorectal excision). This first aspect of the surgery can be done either by conventional surgery or using a laparoscopic or robotic approach, each with its own pros and cons. Conventional surgery in low volume centers has a smaller operative time as compared to laparoscopic approach, and it allows the surgeon direct mobilization and approximation of local tumor spread. Laparoscopic surgery, however, seems to be superior to conventional surgery regarding nerve identification and the use of nerve sparing techniques. Both the laparoscopic and robotic approaches require specialized instruments and have a long learning curve for the surgeon [20].

The intersphincteric groove is entered from the abdomen whenever possible to assess tumor invasion. The perineal part begins with digital and instrumental dilatation followed by the exposure of the anal canal using four to six traction threads (in the absence of a designated retractor). Following exposure, a circular incision is made on the anal mucosa distal to the dentate line—in the event of a total ISR—or at the level of it—for partial ISR. A minimum distance of 1 cm distally was maintained in all cases. The perineal phase continues with intersphincteric circumferential cranial preparation to meet the dissection plane from the abdomen. Following the completion of the dissection, the rectum is delivered through the anus with the transection of the sigmoid colon at the appropriate level. The final part of the surgery consists of a hand-sewn coloanal anastomosis.

2.3. Preoperative staging and preparation

Preoperative investigations consisted of a standard rectal touch, tumor biopsy with a malignant histopathology report. Preoperative imaging consisted of MR in most of cases and computed tomography in some of them and showed stage T2 tumors in all patients (tumor confinement to the rectal wall). Abdominal ultrasound and standard chest X-ray was also routinely performed to further asses the presence of distant metastatic disease.

All patients received long-term pelvic neoadjuvant radiotherapy with a total dose of 50 Gy for 5 weeks according to NCCN Guidelines, V2 and none of them received preoperative chemotherapy. From the entire series, 10 patients showed a type III inferior rectal tumor (intra-anal) and 30 had type II tumors (juxta-anal). From an antigen point of view, CEA and CA 19-9 levels were elevated in all cases and no signs of distant metastatic disease were found on preoperative imaging.

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2.4. Preoperative sphincter function

We chose case series to evaluate the sphincter function using the Wexner score (**Figure 2**). There are numerous ways to evaluate the continence (FIQL, RAFST etc.), but we consider the Wexner score to be the easiest to accomplish and having the best correlation between the patient's perception of continence and the clinical assessment of the surgeon [22–24].

In our case series, we performed preoperative evaluation of the Wexner score in all patients followed by postradiotherapy evaluation. The Wexner score was calculated again at 3, 6 and 12 months following surgery to assess the continence. The medium value of the preradiotherapy Wexner score was **7.65** and the median value for the preoperative (post neoadjuvant radiotherapy) was **4.9**.

2.5. Surgery

All surgical procedures were performed by the same surgeon, 33 partial intersphincteric resections and 7 total intersphincteric resections for type II and III rectal tumors following the technique described above. In five cases, a partial laparoscopic approach was used, and in two cases, full laparoscopic surgery was performed.

Restoration of bowel continuity was achieved by performing a hand-sewn coloanal anastomosis with absorbable threads in all cases, without a protection ileostomy or colostomy. We did not perform a colonic J pouch in any patient. The technique consists of some key points illustrated below (**Figures 3–9**).

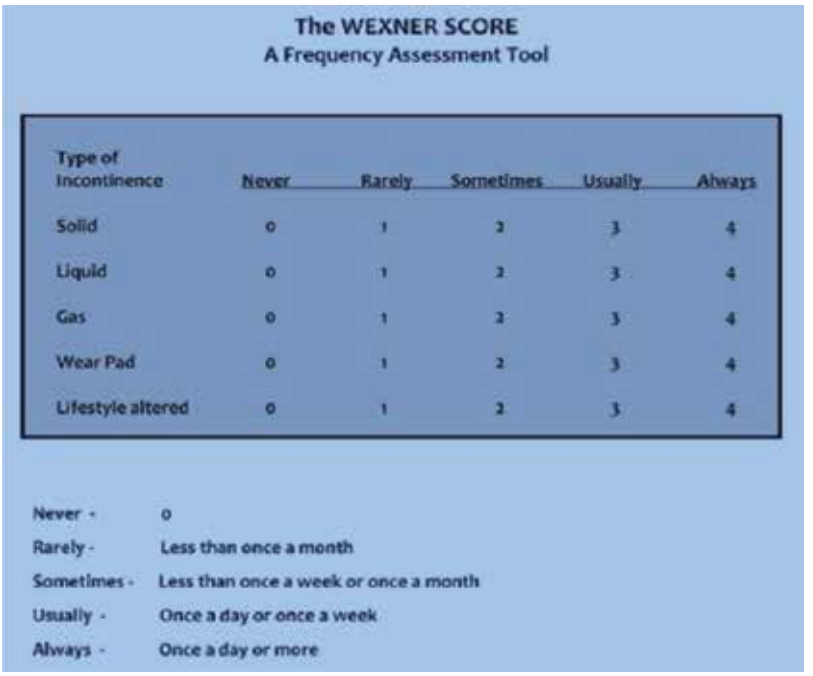

Figure 2. Wexner score system.

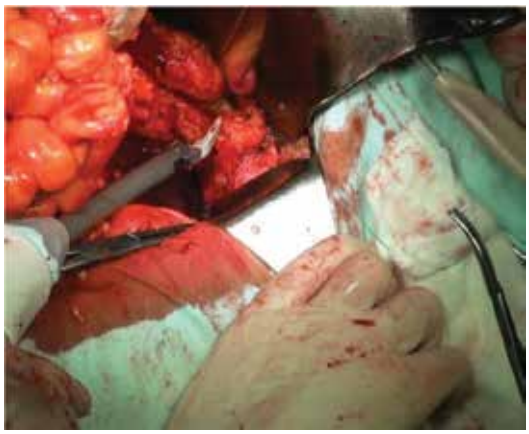

Figure 3. Mobilization of the sigmoid and rectum during the abdominal time.

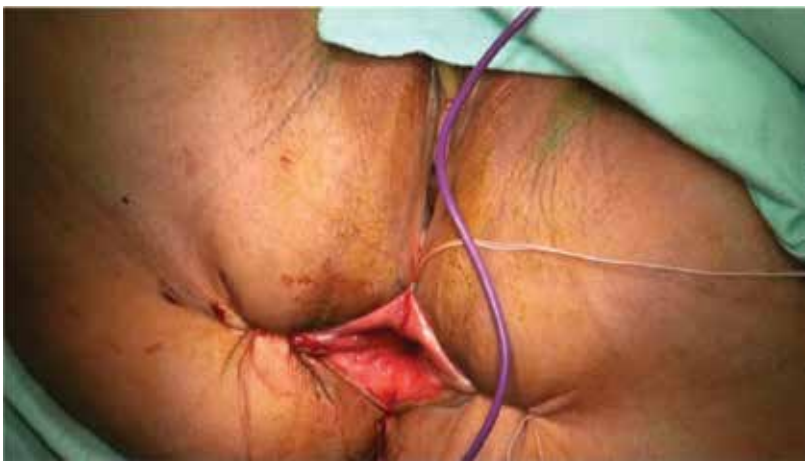

Figure 4. Exposure of the anal canal for the perineal time.

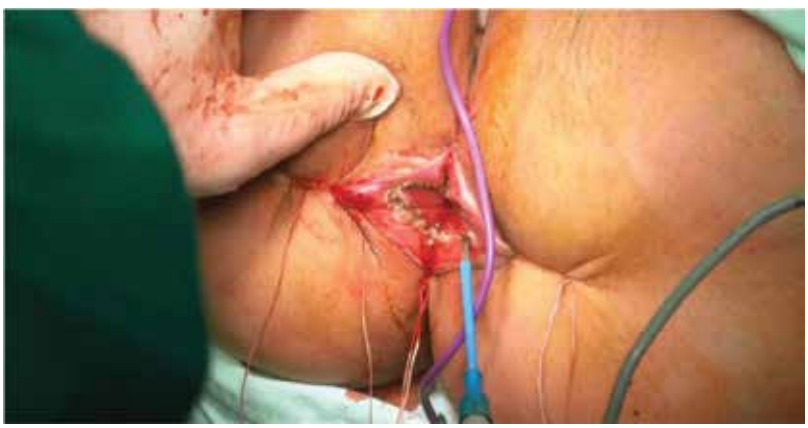

Figure 5. Demarcation of the anal resection line.

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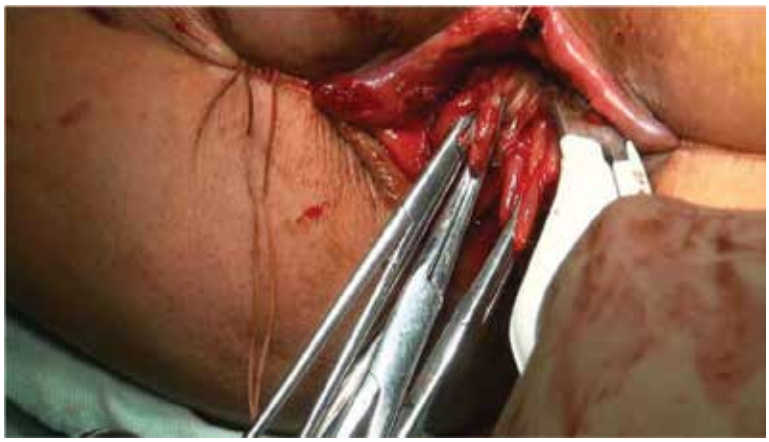

Figure 6. Intersphincteric removal of the anal canal.

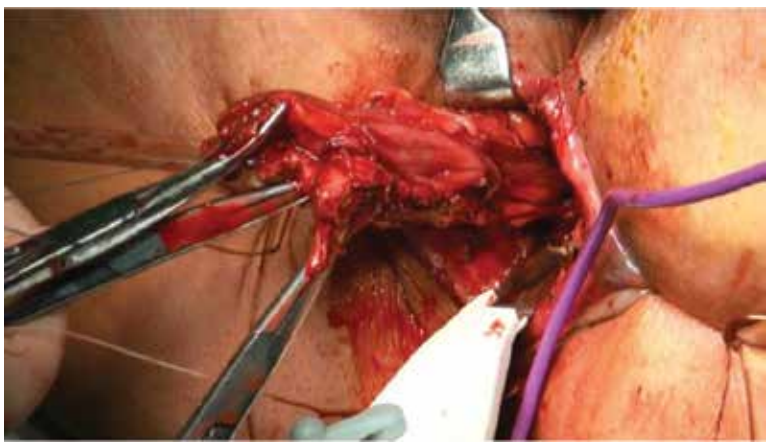

Figure 7. Advancement with the removal of the anal canal.

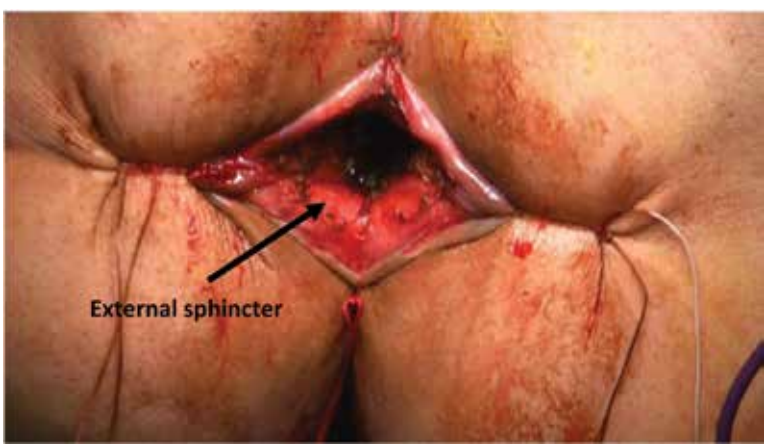

Figure 8. Anal sphincter—final aspect.

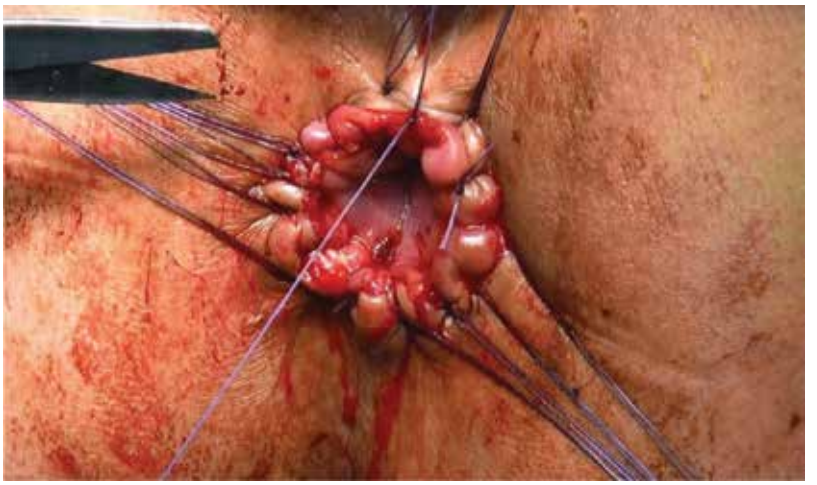

Figure 9. Colo-cutaneous hand-sewn anastomosis.

3. Results

The medium hospital stay was 9 days. Bowel movement resumed on the first or second day following surgery.

There were no perioperative complications and no hospital mortality was present. What we consider a minor complication was noted in the case of five patients at ~10 days following surgery in the form of a muco-submucosal necrosis at the level of the pulled throw colonic segment [25], complication treated in an ambulatory setting, without the need of anesthesia. This complication is probably due to mucosal ischemia. We recorded no wound infections/wound dehiscence or intra-abdominal abscess. None of the patients developed anastomotic leakage or required a second surgery.

At 3 months after surgery, patients had no signs of local tumor recurrence on clinical exam and imaging (MR). CEA and CA 19-9 levels were still elevated in the case of 22 patients 3 months following surgery. The Wexner score obtained at this time showed a median value of **13.2**. The patients subjectively reported a relatively unsatisfactory continence especially in the case of gas.

At 6 months following surgery, only three patients had elevated CEA and CA 19-9 levels. Clinical exam and imaging invalidated the presence of local relapse or the existence of metastatic disease except for one case that presented with sacrum local recurrence (**Figure 10**) that required removal. The Wexner score obtained at this time showed a median value of **9.7**. Subjectively, patients reported a satisfactory continence with few episodes/week of especially gas incontinence.

At 12 months postoperative all patients showed normal CEA and CA 19-9 values. Clinical exam and follow-up imaging invalidated the presence of local relapse or existence of metastatic

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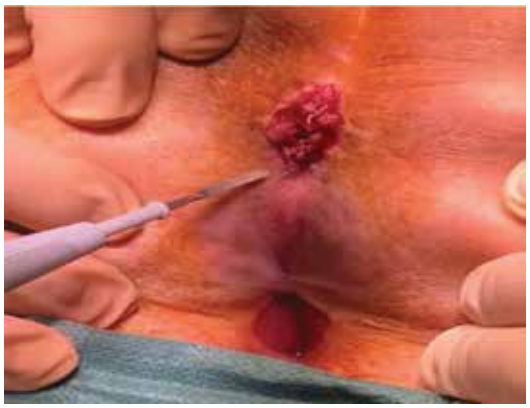

Figure 10. Local recurrence at 6 months following surgery.

disease. The median Wexner score was **8.2** in the case of these patients, and they declared that they are satisfied regarding the choice of surgery and the level of continence, under the terms of major surgery.

4. Discussions

Although intersphincteric resections are relatively "new to the surgeon's arsenal" numerous studies have been published regarding its effectiveness and overall satisfaction of patients following this type of surgery. Hohenberger et al. found that in carcinomas of the lower third of the rectum, the application of abdomino-peranal intersphincteric resection can reduce the need for rectal excision by 20%. Neo-/adjuvant radiochemotherapy is required to reduce locoregional recurrence to an acceptable level [26]. These findings are consistent with our own findings in our relatively small group.

The "father" of intersphincteric resections, Schiessel states that in a study consisting of 121 patients, the technique has satisfactory long-term results not only in functional and oncologic respects, but also states that an important aspect is the preoperative correct staging of cases. Preoperative imaging consisting of MRI, sphincter manometry is mandatory. Case selection is the key to success [27].

5. Conclusions

Intersphincteric resections seem to be a feasible option in highly selected cases with low rectal cancer that refuse the presence of a colostomy bag be it a temporary one.

Oncologic outcome is like that of classic procedures.

Functional outcomes are satisfactory following ISR from the patient's point of view.

Complications found in our small study group are "mild" and seldom met as compared to complications reported in the case of abdominoperineal resections.

Other authors have also evaluated found ISR to be satisfactory from an oncological point of view. In one study, authors report a 3-year disease-free survival in ISR group was 97% and a 5-year disease-free survival was 93% [28].

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Conflict of interest

The authors declare no conflict of interest in preparing this article.

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Limitations of the study

The limitations of this study are represented by the low number of cases, our center not being a colorectal surgery center thus the addressability of the patients is low. Our study is focused predominantly on the use of the Wexner score system for the evaluation of sphincter function, other scoring systems (Rothenberger, Vaizey, Fecal Incontinence Severity Index) could provide a better assessment of the functional outcome, but the easiest score to use is in our opinion the Wexner. The short-term follow-up is another inconvenient of the study, the results being preliminary. Follow-up of these patients is ongoing.

Abbreviations

APR	abdominoperineal resection
WWII	World War Two
GI tract	gastrointestinal tract
ISR	intersphincteric resection

TME	total mesorectal excision
MR	magnetic resonance
CT	computed tomography

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Chapter 8

Actinic Papillary Fibroelastoma of the Left Ventricle

Tomás Francisco Cianciulli and
María Cristina Saccheri

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81024

Abstract

We present the case of a 69-year-old woman with a history of endometrial carcinoma in 1996, who underwent a total hysterectomy and bilateral adnexectomy. The patient also received chemotherapy (doxorubicin and cisplatinum) and local radiotherapy (50 Gy) because of a single lung metastasis, with total remission during later follow-up**.** During follow-up, 10 years later following radiotherapy, a transthoracic echocardiogram (TTE) revealed an image consistent with a primary cardiac tumor (papillary fibroelastoma) or metastatic cardiac tumor on the posteromedial papillary muscle**.** Cardiac magnetic resonance imaging (MRI) revealed a solid mass on the posteromedial papillary muscle with late enhancement, consistent with a primary cardiac tumor. During surgery, the tumor located in the posteromedial papillary muscle was resected. A pathological examination revealed the presence of a tumor mass with a core of dense connective tissue surrounded by a layer of hyperplastic endocardial cells characteristic of a papillary fibroelastoma. After 8 years of follow-up, the patient remains asymptomatic.

Keywords: cardiac tumor, radiotherapy, actinic papillary fibroelastoma, echocardiography, left ventricular mass, malignancy

1. Background

Radiotherapy can affect the heart and lead to effusive and constrictive pericarditis, coronary artery disease, myocardial fibrosis, and valvular disease. Radiation-associated valvular disease can progress over several years from asymptomatic valvar thickening to symptomatic valvular dysfunction. We report the case of a woman, who developed severe mitral regurgitation and an endocardial left ventricular papillary fibroelastoma (PFE) a long time after chest radiotherapy. The mechanism for this pathology is demonstrated, and histological findings are shown.

2. Case report

We present the case of a 69-year-old woman with a history of endometrial carcinoma in 1996, who underwent a total hysterectomy and bilateral adnexectomy. The patient also received chemotherapy (doxorubicin and cisplatinum) and local radiotherapy (50 Gy) because of a single lung metastasis, with total remission during later follow-up. About 10 years later following radiotherapy, she had increasing breathlessness and a new pansystolic murmur at the left sternal edge and progressed insidiously to retrograde left heart failure [1].

An ECG showed a sinus rhythm and complete right bundle branch block. A chest X-ray revealed a normal cardiac contour with upper right pleural thickening with a sequelar appearance, and blood flow redistribution consistent with pulmonary edema. A transthoracic and transesophageal echocardiogram showed thickening and retraction of the mitral valve, severe mitral regurgitation, and severe pulmonary hypertension. The left ventricular function was normal. An assessment of myocardial perfusion with SPECT and coronary angiography were normal. During follow-up, a transthoracic echocardiogram (TTE) revealed an image consistent with a primary or metastatic cardiac tumor on the posteromedial papillary muscle (**Figure 1A**, https://www.dropbox.com/s/q9mtt67rcvps82u/Movies%201%20and%202.rar?dl=1). Cardiac magnetic resonance imaging (MRI) revealed a solid mass on the posteromedial papillary muscle with late enhancement, consistent with a primary cardiac tumor (**Figure 1B**).

Parasternal short-axis view from a transthoracic echocardiogram showing a solid mass on the posteromedial papillary muscle consistent with a primary cardiac tumor (papillary fibroelastoma). A mild pericardial effusion and large left pleural effusion are also visible.

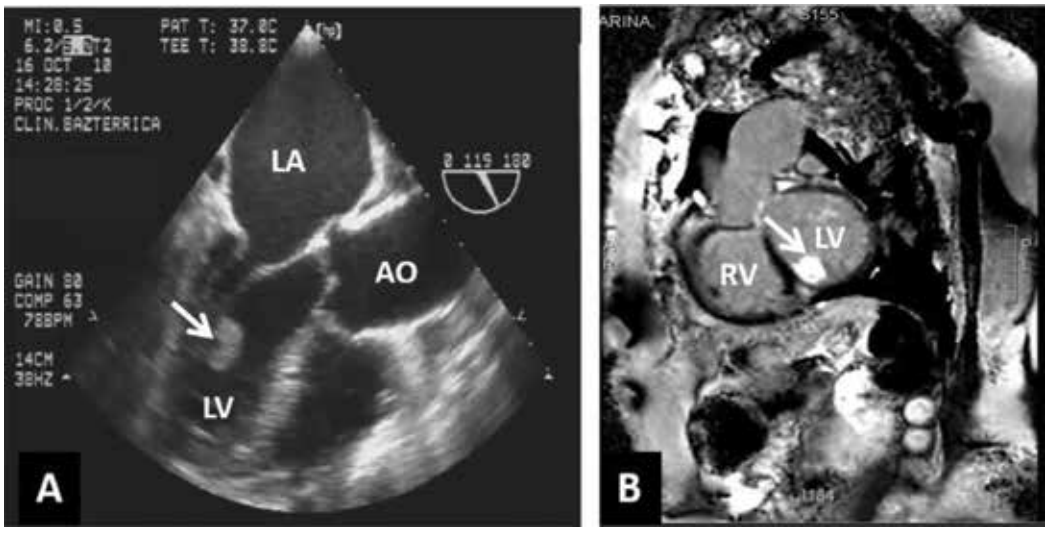

Figure 1. Transesophageal echocardiography. Longitudinal view of the left ventricle at 119° (A) and cardiac MRI (B) shows a solid mass on the posteromedial papillary muscle (arrows), measuring 21.9 × 14.6 mm with gadolinium enhancement consistent with a primary cardiac tumor. LA = left atrium; LV = left ventricle; RV = right atrium; Ao = ascending aorta.

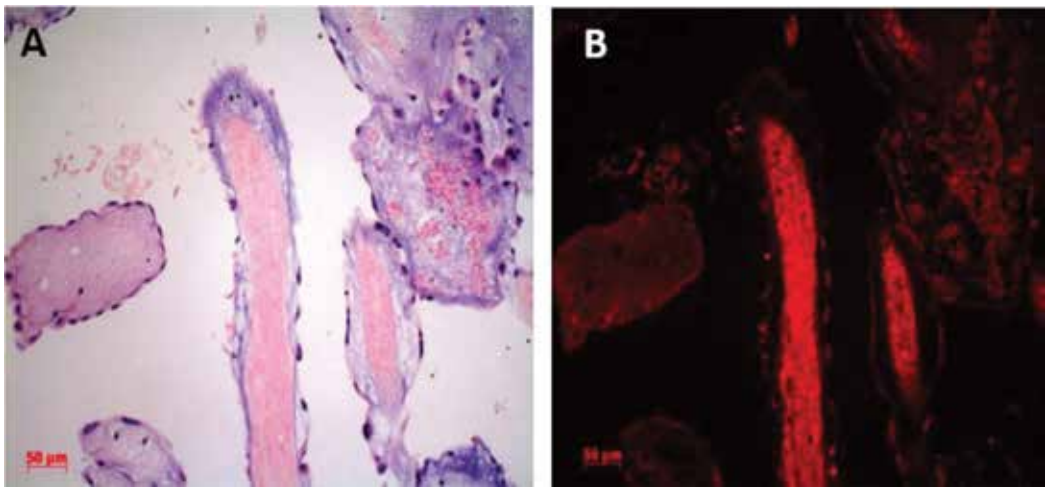

Figure 2. Pathology examination. A: Panoramic image of the papillary fibroelastoma and the villi. B: With fluorescence technique, the same papilla shows an axis of elastic fibers.

During surgery, the mitral valve was thickened, and the patient underwent mitral valve replacement with an SJM #29 biological prosthesis with resection of the tumor located in the posteromedial papillary muscle. During the postoperative period, the retrograde heart failure regressed, pulmonary pressures were normalized, and the patient remained symptom-free. A pathological examination revealed the presence of a tumor mass with a core of dense connective tissue surrounded by a layer of hyperplastic endocardial cells characteristic of a papillary fibroelastoma (**Figure 2**); the mitral valve had signs of radiotherapy-induced damage with increased collagen and dysmorphic nuclei. After 8 years of follow-up, the patient remains asymptomatic.

3. Discussion

Radiosensitivity of the myocardium, pericardium, and great vessels is an issue of great concern. Previously, the heart was thought to be a radio-resistant organ; however, this theory was subsequently abandoned when late cardiac involvement was found in young patients, who had received radiotherapy treatment due to Hodgkin's disease [2].

The prevalence of cardiac disease associated with radiation may clinically manifest after a very prolonged time period, and its incidence is on the rise because of the increasing survival of cancer patients. Modern treatment techniques seem to have decreased the toxicity, but long-term results that allow an assessment of its effects are still lacking. It is worth noting that survivors who do not experience recurrence of the original tumor or develop a second malignant tumor have a greater risk of cardiovascular death than the general population, and in some series that risk is fivefold [3].

Radiation causes progressive and irreversible damage; acute and chronic pericardial manifestations are the most frequent manifestations seen in daily practice. However, valvular lesions,

conduction abnormalities, and cardiomyopathies due to myocardial fibrosis or coronary disease have also been described. International guidelines on cancer management recommend aggressive management of cardiovascular risk factors and performing perfusion exams or echocardiograms at baseline and 10 years after radiation therapy to detect coronary disease and valvular lesions in these patients [4].

In terms of the additional incidental finding of a PFE on the papillary muscle, it should be noted that PFEs are the second most common primary benign cardiac tumor after myxoma, and it most often affects the heart valves, as opposed to myxomas, which are most often found in the atria.

A cardiac MRI with gadolinium showed that the solid mass on the papillary muscle exhibited late enhancement, suggesting that the mass was consistent with a primary cardiac tumor rather than a metastatic lesion. The characteristic irregular surface with mobile papillae observed on the TEE suggested that the tumor was a PFE.

The valves most frequently affected by PFEs are the mitral and aortic valves. In our experience with a total of 54 papillary fibroelastomas [5], in 29.6% (16/54) of patients, they were located in the endocardium of the left ventricle (LV), as in this case. The most common symptoms are due to a peripheral embolism, and the following symptoms have been described: stroke, acute myocardial infarction, and sudden death due to an embolism or obstruction of the coronary ostium. This type of cardiac tumor is not associated with valvular regurgitation. Several authors have reported that this type of tumor is more often seen in elderly patients with longstanding heart disease; therefore, this suggests that they occur due to mechanical damage and involve a degenerative process [6]. In accordance with this theory, in a series of 17 patients with a diagnosis of fibroelastoma, echocardiography was shown to correlate well with pathological findings, and these tumors were usually found in areas of cardiac irritation, such as the aortic and mitral valves [5]. This information proved to be very important in our case, since the damage caused by radiotherapy might have affected the valvular and subvalvular mitral apparatus, thus enhancing the potential development of this type of tumor.

4. Conclusion

We report the case of a patient with radiotherapy-induced symptomatic severe mitral regurgitation and an asymptomatic papillary fibroelastoma, which is a rare complication of thoracic radiotherapy that required valve replacement and resection of the tumor. The latent period between radiotherapy and these complications was 10 years and was related to the dose of radiation received. The increase in survival seen in cancer patients has resulted in an increased frequency of these types of complications. This unusual mechanism of formation of an actinic papillary fibroelastoma should be disseminated among cardiologists. In patients who have survived for long periods of time after radiotherapy, it is important to remember that cardiac complications may indeed occur, and the treating physician is responsible for detecting them.

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This book is a marvelous compendium of eight articles that cover a wide range of topics, including breast cancer: management and survivorship, rectal cancer intersphincteric resection, head and neck cancer diagnosis and radiotherapy, synthetic peptides as antitumor agents, and recent advances in thyroid cancer. It has been a wonderful opportunity to co-edit this special edition. We are greatly appreciative of the work of all the contributors to the book, who brought with them tremendous diversity of perspectives and fields truly reflective of the complexity of the topic and who, through coming together in this project, serve as a nidus of the multidisciplinary collaboration in this field. Finally, we must acknowledge the thousands of cancer patients who have participated in the studies that have provided the information that has advanced the field so greatly in recent years.

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