Chapter 1

Introduction

1.1 The Fight against Cancer

Cancer is a set of diseases characterized by the uncontrolled growth and spread of abnormal cells. Great advances have been made in understanding the underlying biology of cancer [1, 2]. Apart from the discovery of an ever-increasing number of genetic abnomalities and signalling-pathway defects stemming from proliferating cancer cells themselves, recent research has also highlighted the complex interplay between cancer cells and their tumor microenvironment [3]. *In situ* cancer tumors are heterogeneous, consisting not only of cancer cells (which themselves are not homogeneous inside the tumor, as demonstrated by the recent discovery of cancer stem cell populations [4]), but also various types of stromal support cells [5], blood vessels [6], and immune cell populations. How these different components interact and support the growing tumor can greatly affect tumor progression. The large number of potential therapeutic targets within the tumor generated from these discoveries have led to the development of many novel drugs targeting specific components of tumors (figure 1.1) . In particular, nanotherapy, which describes the class of agents with a size range of ~1–100 nm, is a promising drug design strategy that is being actively investigated. Cancer nanotherapies show great promise for oncological treatment, since they can be engineered to overcome barriers of uptake posed by the tumor micronenvironment [7]. Additionally, nanotherapies can reduce treatment-related toxicity by concentrating delivery of drugs into the tumor mass.

Translating this basic biological understanding and engineering progress to effective clinical endpoints (i.e.increased patient survival) remains a challenge. Survival rates from several types of cancer remain low (figure 1.2, [8]). A major issue contributing to this is the fact that our un-

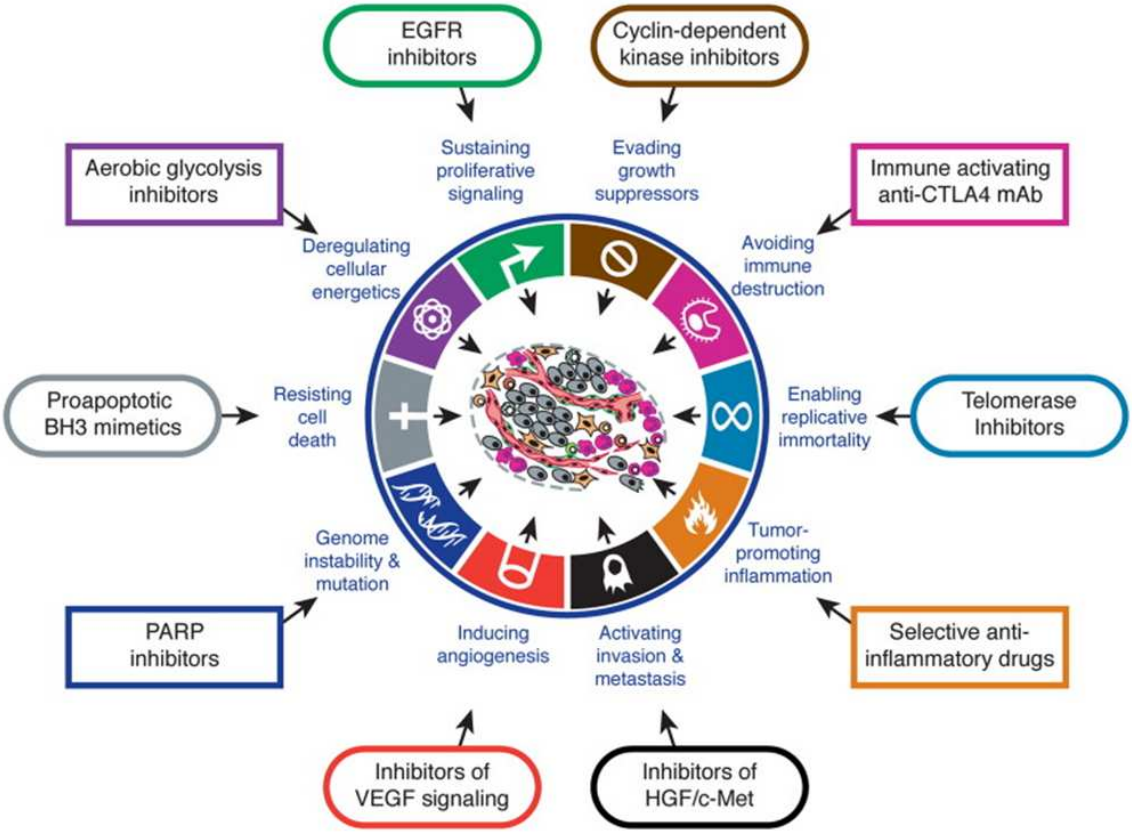

Figure 1.1: Therapeutics targeting different aspects of cancer growth are under development (adapted from [1]).

derstanding and evaluation of tumor progression and response to treatment in individual patients remain limited. For example, consider a tumor type studied in this work, Burkitt's lymphoma (BL). The genetic profiles of well-defined BL are known; however patients often present a phenotype intermediate between BL and other aggressive lymphomas, making diagnosis difficult [9]. Although the disease has been shown to be quite chemosensitve, no concensus treatment exists [10]. Often, a high-intensity chemotherapy regimen is administered to the patient over 3–6 cycles, which can take 3–6 months to complete. Treatment evaluation is then documented 4–8 weeks posttreatment. The criteria for treatment response is based mainly on anatomical reduction of tumor burden, complemented by laboratory tests and tissue biopsies to confirm response *after* completion of therapy [11]. While recent response rates are quite good, even close to 100%, some variants of BL in adolescents and young adults have poor prognosis. If cancer nanotherapy is to be successfully translated to the clinic to treat diseases such as BL, tools that enable us to understand the causes of poor tumor treatment response and identify individual patients that do not respond well early in the treatment regimen will be highly desirable. noninvasive molecular imaging is one such tool.

1.2 Personalized Non Invasive Molecular Imaging to Improve Cancer Treatment

Noninvasive imaging has become indispensible for both research and clinical management of cancer during the past 20–30 years. Visualization of the tumor mass with high-resolution anatomical imaging modalities such as x-ray, computer tomography (CT), and magnetic resonance imaging (MRI) enables cancer diagnosis and staging. This has been augmented by the availability of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) to locate tumors. These imaging modalties now play a vital role in forming the criteria for tumor response [11].

Advances in the field of molecular imaging are enabling researchers to address questions about the complex dynamics between various components of the tumor and the therapies used to treat them. Studies with the imaging modalities mentioned above are moving beyond mere size measurement and tumor staging. Imaging techniques and imaging probes are being developed to monitor specific molecular aspects of tumor growth [12]. The imaging modalities mentioned are noninva-

Five-year Relative Survival Rates* (%) by Stage at Diagnosis, 2001-2007

	All Stages	Local	Regional	Distant		All Stages	Local	Regional	Distant
Breast (female)	89	99	84	23	Ovary	44	92	72	27
Colon & rectum	64	90	69	12	Pancreas	6	22	9	2
Esophagus	17	37	18	3	Prostate	99	100	100	29
Kidney†	70	91	63	11	Stomach	26	62	28	4
Larynx	61	77	42	33	Testis	95	99	96	73
Liver‡	14	27	9	4	Thyroid	97	100	97	56
Lung & bronchus	16	52	24	4	Urinary bladder§	78	71	35	5
Melanoma of the skin	91	98	61	15	Uterine cervix	69	91	57	19
Oral cavity & pharynx	61	82	56	34	Uterine corpus	82	96	67	16

*Rates are adjusted for normal life expectancy and are based on cases diagnosed in the SEER 17 areas from 2001-2007, followed through 2008.
†Includes renal pelvis. ‡Includes intrahepatic bile duct. §Rate for in situ cases is 97%.

Local: an invasive malignant cancer confined entirely to the organ of origin. **Regional:** a malignant cancer that 1) has extended beyond the limits of the organ of origin directly into surrounding organs or tissues; 2) involves regional lymph nodes by way of lymphatic system; or 3) has both regional extension and involvement of regional lymph nodes. **Distant:** a malignant cancer that has spread to parts of the body remote from the primary tumor either by direct extension or by discontinuous metastasis to distant organs, tissues, or via the lymphatic system to distant lymph nodes.

Source: Howlader N, Krapcho M, Neyman N, et al. (eds). *SEER Cancer Statistics Review, 1975-2008*, National Cancer Institute, Bethesda, MD, www.seer.cancer.gov/csr/1975_2008/, 2011.

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(a) Five year cancer survival rates, stratified by stage at diagnosis.

Trends in 5-year Relative Survival Rates* (%) by Race, US, 1975-2007

	All races			White			African American		
	1975-77	1987-89	2001-2007	1975-77	1987-89	2001-2007	1975-77	1987-89	2001-2007
All sites	49	56	67†	50	57	69†	39	43	59†
Brain	22	29	35†	22	28	34†	25	31	40†
Breast (female)	75	84	90†	76	85	91†	62	71	77†
Colon	51	60	65†	51	61	67†	45	53	55†
Esophagus	5	10	19†	6	11	20†	3	7	13†
Hodgkin lymphoma	72	79	86†	72	80	88†	70	72	81†
Kidney & renal pelvis	50	57	71†	50	57	71†	49	55	68†
Larynx	66	66	63†	67	67	65	59	56	52
Leukemia	34	43	57†	35	44	57†	33	36	50†
Liver & intrahepatic bile duct	3	5	15†	3	6	15†	2	3	10†
Lung & bronchus	12	13	16†	12	13	17†	11	11	13†
Melanoma of the skin	82	88	93†	82	88	93†	58‡	79‡	73‡
Myeloma	25	28	41†	25	27	42†	30	30	41†
Non-Hodgkin lymphoma	47	51	70†	47	52	71†	48	46	62†
Oral cavity & pharynx	53	54	63†	54	56	65†	36	34	45†
Ovary	36	38	44†	35	38	43†	42	34	36
Pancreas	2	4	6†	3	3	6†	2	6	4†
Prostate	68	83	100†	69	85	100†	61	72	98†
Rectum	48	58	68†	48	59	69†	45	52	61†
Stomach	15	20	27†	14	19	26†	16	19	27†
Testis	83	95	96†	83	95	97†	73‡#	88‡	86
Thyroid	92	95	97†	92	94	98†	90	92	95
Urinary bladder	73	79	80†	74	80	81†	50	63	64†
Uterine cervix	69	70	69	70	73	70	65	57	61
Uterine corpus	87	83	83†	88	84	85†	60	57	61

*Survival rates are adjusted for normal life expectancy and are based on cases diagnosed in the SEER 9 areas from 1975-77, 1987-89, and 2001 to 2007, and followed through 2008. †The difference in rates between 1975-1977 and 2001-2007 is statistically significant ($p < 0.05$). ‡The standard error is between 5 and 10 percentage points. #Survival rate is for cases diagnosed in 1978-1980.

Source: Howlader N, Krapcho M, Neyman N, et al. (eds). *SEER Cancer Statistics Review, 1975-2008*, National Cancer Institute, Bethesda, MD. seer.cancer.gov/csr/1975_2008/, 2011.

American Cancer Society, Surveillance Research, 2012

(b) Five year cancer survival rates, stratified by race.

Figure 1.2: Five year cancer survival rates in the United States, 2001–2007. Adapted from [8].

sive, allowing real-time studies of the intact tumor during growth and/or therapy. They are also quantitative, which enable comparisons intra- and intersubjects across multiple time points. There is also increasing recognition that studies integrating information from multiple modalities can be synergistic, providing information not available in unimodal situations. The most spectacular example of this synergy is the widespread adoption of PET/CT systems. CT gives high-resolution anatomical context to the PET data, and the combination has demonstrated clear clinical benefit [13]. Recent developments in hybrid PET/MRI promises to offer even more synergistic value [14]. MRI not only provides high-resolution anatomical images, but can also provide functional information that is complementary to PET. The field of hybrid PET/MRI is still in its infancy; continued development of PET/MRI technology is necessary to realize its full potential.

Progress in the design and development of cancer nanotherapies are informed by their interactions with the tumor microstructure *in situ*. Most investigations to understand the interaction of nanotherapies and the tumor microenvironment are done *in vitro* [15, 16], *ex vivo* [17], or invasively [18], which may not always simulate *in situ* situations well. Noninvasive imaging modalities, such as PET/MRI, will be very useful to study cancer nanotherapy uptake and response. Researchers have begun to use noninvasive imaging to study the uptake of nanotherapies, mostly focusing on examining the gross uptake of therapies into the tumor mass [19, 20].

The goal of this thesis is to develop clinically applicable, noninvasive imaging technologies, specifically PET and MRI, to understand the uptake and response of cancer nanotherapies beyond simple tumor size measurements and the gross uptake of nanotherapies into tumors. We first describe the development and evaluation of a small animal hybrid PET/MRI system to study the heterogeneous uptake of antibodies into the tumor. The second part of the thesis concerns the development of a functional imaging technique, diffusion MRI, to model and follow the early response of a nanotherapy currently in clinical trials. The final part of the thesis describes work to develop PET/MRI methods to study the effects of the tumor modulating peptide iRGD, which has the potential to significantly improve the uptake of nanotherapies into the tumor.

1.3 Outline and Scope of Thesis

This thesis describes work developing and validating a first-generation small animal, simultaneous PET/MRI imaging system and the application of both PET and MRI (individually or together) techniques to study the uptake and response of different targeted cancer nanotherapies.

1.3.1 Review of Targeted Cancer Nanotherapies, Tumor-modulating Peptides and Multimodal Noninvasive Imaging

Chapter 2 first reviews the current status of targeted nanotherapies for cancer. We describe the different design approaches being investigated to improve targeting of drugs into the tumor site. Tumor microenvironmental factors which may affect the kinetics and biological activity of targeted therapies are described. Next, we review recent attempts to improve tumor drug uptake by modulating the tumor microenvironment. Treatments which attenuate barriers to drug access in the tumor may act synergistically with carefully designed targeted therapies to enhance therapeutic response.

The second half of the chapter reviews the application of noninvasive imaging to study cancer therapy uptake and tumor response. The basic physics of PET, MRI, and DWBA, as well as their use in imaging cancer nanotherapy are reviewed. Advances in the nascent field of hybrid PET/MRI imaging are outlined.

1.3.2 Developing Simultaneous PET/MRI for Robust *In Vivo* Studies

Hybrid PET/MRI systems are being built. However, thorough characterization and verfication of such systems are required before being used in meaningful *in vivo* studies. Chapter 3 describes the characterization and development of a first-generation small animal PET/MRI system to enable robust physiological studies. First, we examine potential interference effects of the PET insert on MRI image quality with direct regards to *in vivo* studies that we wish to pursue. Next, we present work on the development of a reliable image coregistration strategies between simultaneously acquired PET and MRI images.

Making believable physiological inferences from high-resolution PET and MRI datasets requires that the images are faithful to real contrast distributions in the imaged tissue. We describe

work that validated the imaging ability of the PET/MRI system to reveal intratumoral uptake patterns as compared to a commercial PET system and DWBA.

To enable robust physiologyical studies, complete integration of the PET and MRI hardware and software is necessary. In the last part of the chapter we describe our efforts to streamline the hybrid PET/MRI system technology and consider MRI interference effects on the PET insert that need to be addressed in next generation systems.

1.3.3 Serial Diffusion MRI to Monitor and Model Early Treatment Response to the Targeted Nanotherapy CRLX101

Successful translation of targeted cancer nanotherapies to the clinic requires robust tools to evaluate effective patient response. noninvasive imaging techniques show great promise in this regard and can be directly translated from preclinical studies to clinical trials. Chapter 4 describes work on the evaluation of a functional MRI method, diffusion MRI, to monitor the response of CRLX101 in a preclinical murine model of Burkitt's lymphoma. We also applied the diffusion MRI data to a logistical model of tumor growth to evaluate the model's ability to predict CRLX101's activity.

1.3.4 Imaging the Tumor Response to the Tumor-penetrating Peptide iRGD

IRGD is a tumor-penetrating peptide that has been shown to improve tumor delivery of drugs by modulating the tumor microenvironment. Translation of this peptide to the clinic requires a robust assay of its efficacy. Chapter 5 describes preliminary results in developing noninvasive (PET and MRI) assays of iRGD response.

1.3.5 Summary and future work

Future directions raised by the current work are presented in chapter 6.

Additional information and studies related to work described in this thesis are presented in the appendices.