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Cancer Therapy Evolution: When Genetics and Epigenetics Intertwine to Create Novel Opportunities

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Abstract

In ancient medical handbooks, Hippocrates and Galen declared cancer as an incurable disease. Since Greek antiquity this two minds have shaped the current practice of medicine and their grim statement about cancer therapy remains a major challenge for our species in the 21 century. Our increasing understanding of cancer biology has led to the development of molecularly targeted anticancer drugs. The promising outcomes of targeted therapies and the incremental improvements in patients' survival have given hope for a complete cancer remission. Unfortunately, targeted therapies are currently facing the presence of tumour resistance, often resulting from compensatory signalling pathways, or from the development of acquired resistance in cancer cells *via* clonal evolution under the selective pressures of treatment. Exploring the role of tumour heterogeneity in the development of drug resistance lead to a new perception of cancer as a complex, dynamic and adaptive ecosystem underpinned by genetic diversity and epigenetic plasticity. Despite this negative aspect, inherent Darwinian character of cancer cells alternatively paves the way towards novel opportunities for the development of revolutionary cancer therapies.

Introduction

In ancient Greek civilization, cancer treatments were based in the used of medicines such as extracts from chickpea, adderwort, stinging nettle, and other plants [1]. Surgical approaches accompanied by blood-letting have been described as early as the first century A.D., [1]. The first revolution in cancer therapy occurred in the middle of the 20th century when a correlation between mustard gas exposure and depletion of lymphocytes in the blood of soldiers during World War II was observed [2-4]. This prompted the hypothesis that nitrogen mustard compounds could be used to inhibit the growth of cancerous white blood cells in leukaemia and lymphomas. At the same time, a study reported the potential of folic to acid accelerate the growth of leukaemia cells. Subsequently, clinical trials involving methotrexate, a folate antagonist, to treat leukaemia were implemented [2,5]. In 1903, radiation therapy, initially applied as palliative care, was found to improve patients' survival [6]. Since then, treatments based on either radiation therapy, or chemotherapy became classical approaches against cancer. However, both of these traditional methods are crude as they kill many normal cells, leading to side effects and can ultimately result in more aggressive cancers.

Consequently, this led us to the development of targeted therapies that are designed to fight cancer cells with more precision and potentially fewer side effects. These therapies specifically interfere with signalling pathways involved in cancer progression. Indeed, more detailed understanding of tumour biology revealed that each individual tumour accumulates loads of genomic and epigenetic alterations during cancer evolution. These alterations are translated by molecules that can be further targeted by a growing arsenal of drugs.

The present review aims at giving a comprehensive view of the current advances in anti-cancer targeted therapies. We will discuss their clinical potential and explore how cancer genetics and epigenetics contribute to cancer progression and influence tumour response to targeted therapies. Importantly, we will discuss the role of clonal diversity in the development of drug resistance. Eventually we will expose how our understanding of the inherent Darwinian character of cancer cells gives rise to a next generation of evolutionary cancer therapies.

Approved Cancer Targeted Therapies

Hallmarks of cancer initially comprise sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis [7]. Conceptual progress in the field added two additional cancer hallmarks, reprogramming of energy metabolism and evading immune destruction [7]. In addition to cancer cells, tumours exhibit another dimension of complexity as they contain a repertoire of recruited normal cells to creating a real tumour microenvironment [7]. Targeted therapies are drugs

design to interfere with specific molecules underlying different cancer hallmarks (Figure 1). Currently, targeted therapies arsenal goes from small relatively simple molecules, such as tyrosine kinase inhibitors (TKIs) or interfering RNA molecules, to highly complex engineer weapons, such as monoclonal antibodies (mAbs), CAR T cells and vaccines.

TKIs as targeted therapies

Tyrosine kinases catalyse the transfer of a phosphate from ATP to tyrosine residues of a tyrosine-kinase receptor, leading to an activation cascade of molecules involved in cell growth, proliferation, migration and angiogenesis. Inappropriate kinase activity is an important pathway through which cells become cancerous. Small molecule inhibitors (SMIs) can competitively bind to the ATP binding site of a tyrosine kinase, preventing a deregulated activation of downstream signalling during cancer progression (Figure 1). Tyrosine kinase receptors such as *EGFR*, *HER2*/neu and VEGF are classic targets for TKIs.

The early 2000s saw the success of SMIs with 41 US Food and Drug Administration (FDA)-approved SMIs (Table 1). Imatinib was one of the first to receive the approval for chronic myelogenous leukaemia (CML) [8]. This SMI inhibits a constitutive active tyrosine kinase that results from the aberrant fusion of BCR and ABL genes and is at the origin of the development of different leukaemia. Because this fusion occurs in nearly all CML cases, imatinib therapy resulted in a complete hematologic response in 98% of patients [9,10]. Subsequently, CML patients who developed a resistance to imatinib were given dasatinib, another SMI with a boarder range of tyrosine kinase targets [11,12].

Interfering RNA molecules as targeted therapies

Small interfering RNAs (siRNAs), as potent tools for target-specific gene silencing through RNAi, were first observed in 1998 by Craig Mello [13]. Since then, three siRNAs used as cancer targeted therapies received an FDA approval to initiate phase I clinical trials [14]. ALN-VSP comprises two siRNAs that simultaneously target VEGF and KSP genes [15]. CALAA-01 is a tumour inhibitor that targets a protein involved in DNA replication and cell division in several cancers [16-18]. Finally, the Atu027 compound displays RNAi-mediated suppression of protein kinase N3 (*PKN3*) gene expression in vascular endothelial cells. The *PKN3* target gene is a critical factor for cancer progression and metastasis [19] (Figure 1).

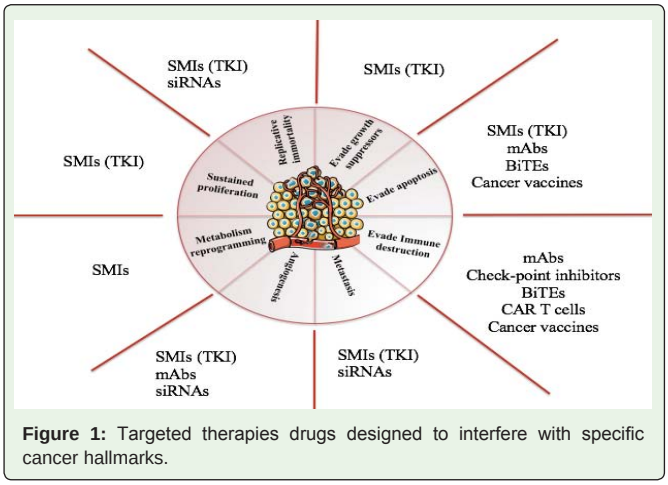

Figure 1: Targeted therapies drugs designed to interfere with specific cancer hallmarks.

In spite of the tremendous potential of RNA-based therapies, there are challenges to bear in mind. RNAs are inherently unstable, and therefore difficult to deliver in high enough amounts to the target tissue due to clearance by the renal system and degradation by nucleases in the blood stream [20,21]. In addition, toxicity due to off-target effects and activation of the immune system are also pressing concerns [20,22].

Monoclonal antibodies as targeted therapies

Monoclonal antibodies (mAbs) are immunoglobulin structures designed to target specific antigens found on the surface of cancer cell but also host cells. Targeted antigens include proteins associated with growth and differentiation, inhibitory molecules (immune checkpoints) or adhesion factors. Their anti-tumour efficacy relies on three main mechanisms. The first one directly induces tumour cell death by inhibiting tumour cell survival signalling and inducing apoptosis. The second aims at disrupting stromal interactions or vascularisation, thus depriving tumours of stable networks and blood nutrients (e.g. anti-VEGF, anti-VEGFR). The third uses anti-tumour immunity to kill cancer cells (Figure 1). For instance, mAbs can target inhibitory molecules involved in host T cell dysfunction to reactivate their anti-tumour activity (e.g. anti-PD-1, anti-PD-L1, anti-CTLA4...). These checkpoint-inhibiting antibodies were a revolution in the field of targeted therapies with anti-PD1 antibody currently approved for the treatment of 7 different malignancies (Table 2). Moreover, anti-PD1 and anti-CTLA-4 are being systematically applied in clinical trials of particular cancer types [23-25]. To date more than 30 mAbs are FDA-approved in the treatment of several cancers and are summarised in Table 2. Importantly, these immune-modulating therapies are used either alone or in combination with each other to potentiate their efficacy [26,27].

However, such combinations also tend to come with more severe side effects [28]. As a consequence, and to reduce the cost of the treatment, bispecific antibodies (bsAb) have recently emerged as potent substitutes to combined anti-cancer therapies. bsAb are genetically engineered antibodies that associate the specificities of two or more antibodies to simultaneously target different antigens. The idea of bsAb emerged in the late 1980s, when Bevan et al. suggested for the first time the use of hybrid antibodies to redirect T cell to attack and kill tumour cells (Figure 1) [29]. Bispecific T-cell Engagers (BiTEs) are bsAbs obtained by the fusion of single-chain variable fragments (scFvs) targeting a tumour-associated antigen and the CD3 subunit of T cell receptor (TCR) [30]. Such construction creates a link between antigen-positive tumour cells and CD3+ T cells in order to force T cells to proliferate and exert their anti-tumour activity. Blinatumomab, was the first BiTE FDA-approved in 2014 for the treatment of acute lymphoblastic leukaemia (ALL) [30]. In a phase III trial conducted in patients with relapsed/refractory B-cell precursor ALL, 44% of blinatumomab-treated patients responded to the treatment. The median overall survival was 7.7 months compared to 4.0 months in standard-of-care chemotherapy group [31].

CAR T cells and their next generation

Therapeutic T cell engineering has recently garnered widespread interest in the field of targeted therapies because of the success of

Table 1: Small molecules inhibitors approved by the FDA for the treatment of cancer.

Inhibitors	Target(s)	FDA-approved indication(s)
Afatinib (Gilotrif)	EGFR (HER1/ RBB1), HER2 (ERBB2/ neu)	Non-small cell lung cancer (with *EGFR* exon 19 deletions or exon 21 substitution (L858R) mutations
Alectinib (Alecensa)	ALK	Non-small cell lung cancer (with *ALK* fusion)
Axitinib (Inlyta)	KIT, PDGFRβ, VEGFR1/2/3	Renal cell carcinoma
Bortezomib (Velcade)	Proteasome	• Multiple myeloma • Mantle cell lymphoma
Bosutinib (Bosulif)	ABL	Chronic myelogenous leukemia (Philadelphia chromosome positive)
Brigatinib (Alunbrig)	ALK	Non-small cell lung cancer (*ALK*+)
Cabozantinib (Cabometyx [tablet], Cometriq [capsule])	FLT3, KIT, MET, RET, VEGFR2	• Medullary thyroid cancer • Renal cell carcinoma
Carfilzomib (Kyprolis)	Proteasome	Multiple myeloma
Ceritinib (Zykadia)	ALK	Non-small cell lung cancer (with *ALK* fusion)
Cobimetinib (Cotellic)	MEK	Melanoma (with *BRAF* V600E or V600K mutation)
Crizotinib (Xalkori)	ALK, MET, ROS1	Non-small cell lung cancer (with *ALK* fusion or *ROS1* gene alteration)
Dabrafenib (Tafinlar)	BRAF	• Melanoma (with *BRAF* V600 mutation) • Non-small cell lung cancer (with *BRAF* V600E mutation)
Dasatinib (Sprycel)	ABL	• Chronic myelogenous leukemia (Philadelphia chromosome positive) • Acute lymphoblastic leukemia (Philadelphia chromosome positive)
Enasidenib (Idhifa)	IDH2	Acute myeloid leukemia (with *IDH2* mutation)
Erlotinib (Tarceva)	EGFR (HER1/ERBB1)	• Non-small cell lung cancer (with *EGFR* exon 19 deletions or exon 21 substitution (L858R) mutation) • Pancreatic cancer
Gefitinib (Iressa)	EGFR (HER1/ERBB1)	Non-small cell lung cancer (with *EGFR* exon 19 deletions or exon 21 substitution (L858R) mutation)
Ibrutinib (Imbruvica)	BTK	• Mantle cell lymphoma • Chronic lymphocytic leukemia • Waldenstrom's macroglobulinemia
Idelalisib (Zydelig)	PI3Kδ	• Chronic lymphocytic leukemia • Follicular B-cell non-Hodgkin lymphoma • Small lymphocytic lymphoma
Imatinib (Gleevec)	KIT, PDGFR, ABL	• GI stromal tumor (*KIT*+) • Dermatofibrosarcoma protuberans • Multiple hematologic malignancies including Philadelphia chromosome-positive ALL and CML
Ixazomib (Ninlaro)	Proteasome	Multiple Myeloma
Lapatinib (Tykerb)	HER2 (ERBB2/neu), EGFR (HER1/ ERBB1)	Breast cancer (*HER2*+)
Lenvatinib (Lenvima)	VEGFR2	• Renal cell carcinoma • Thyroid cancer
Neratinib (Nerlynx)	HER2 (ERBB2/neu)	Breast cancer (*HER2* overexpressed/amplified)
Nilotinib (Tasigna)	ABL	Chronic myelogenous leukemia (Philadelphia chromosome positive)
Niraparib (Zejula)	PARP	• Ovarian cancer • Fallopian tube cancer • Peritoneal cancer
Olaparib (Lynparza)	PARP	Ovarian cancer (with *BRCA* mutation)
Osimertinib (Tagrisso)	EGFR	Non-small cell lung cancer (with *EGFR* T790M mutation)
Palbociclib (Ibrance)	CDK4, CDK6	Breast cancer (*HR+, HER2-*)
Pazopanib (Votrient)	VEGFR, PDGFR, KIT	Renal cell carcinoma
Ponatinib (Iclusig)	ABL, FGFR1-3, FLT3, VEGFR2	• Chronic myelogenous leukaemia • Acute lymphoblastic leukaemia (Philadelphia chromosome positive)
Regorafenib (Stivarga)	KIT, PDGFRβ, RAF, RET, VEGFR1/2/3	• Colorectal cancer • Gastrointestinal stromal tumours • Hepatocellular carcinoma
Ribociclib (Kisqali)	CDK4, CDK6	Breast cancer (*HR+, HER2-*)
Rucaparib (Rubraca)	PARP	Ovarian cancer (with *BRCA* mutation)
Ruxolitinib (Jakafi)	JAK1/2	Myelofibrosis

Sonidegib (Odomzo)	Smoothened	Basal cell carcinoma
Sorafenib (Nexavar)	VEGFR, PDGFR, KIT, RAF	• Hepatocellular carcinoma • Renal cell carcinoma • Thyroid carcinoma
Tofacitinib (Xeljanz)	JAK3	Rheumatoid arthritis
Trametinib (Mekinist)	MEK	• Melanoma (with *BRAF* V600 mutation) • Non-small cell lung cancer (with *BRAF* V600E mutation)
Vandetanib (Caprelsa)	EGFR (HER1/ERBB1), RET, VEGFR2	Medullary thyroid cancer
Vemurafenib (Zelboraf)	BRAF	Melanoma (with *BRAF* V600 mutation)
Vismodegib (Erivedge)	PTCH, Smoothened	Basal cell carcinoma

CD19 chimeric antigen receptor (CAR) therapy [32]. CARs are synthetic cell receptors for antigen that are genetically introduced into T cells to increase their avidity and reproducibility [33]. CARs integrate a single chain variable fragment (scFv) of a specific antibody and a signaling domain CD3ζ to generate T cells that will attack cancer cells under the guidance of the CAR specificity [33,34] (Figure 1). CARs targeting CD19, a cell surface molecule found in most leukaemia and lymphomas, have yielded high remission rates in patients with chemo-refractory and relapsed disease, including ALL, CML, and non-Hodgkin lymphoma [32].

However, when CAR-T cells successfully drive tumour regression, a major drawback lies in severe adverse effects mainly caused by a cytokine release syndrome (CRS) related to excessive activation of these cells [32-34]. Another weakness is the short persistence of conventional CAR-T cells. Due to strong and lasting TCR/CAR cell surface expression, CAR-T cells are constantly sollicitated, which drives their exhaustion and terminal differentiation more rapidly [35]. To further enhance the efficacy and safety of CAR-T cells, some strategies were reported such as inclusion of suicide gene or new engineering modalities that target nucleases like CRISPR [32,36]. In this last context, CRISPR/Cas9 method is used to decrease the level of endogenous TCR by targeting CARs to the T-Cell Receptor Alpha Constant (TRAC) locus, while CAR is expressed under the promoter of an endogenous gene to enhance its stability and reproducibility [35]. In contrast to conventional CAR-T cell, such construction was reported to generate a bulk of long-term memory effector CAR-T cells. Furthermore, TRAC-CAR T cells express lower levels of inhibitory receptors (like PD-1, TIM-3 and LAG3), which prevent the triggering of early T cell exhaustion, and allow long-lasting control of murine hematopoietic tumour cells [35]. Whether TRAC-CART cells are clinically efficient over conventional CAR T cell therapy and reduce CRS side effect deserve further investigations.

Anticancer vaccines

Cancer vaccine can be either therapeutic or prophylactic. Therapeutic cancer vaccines usually utilise tumour-associated antigens to stimulate specific T cells and drive cancer cell killing [37] (Figure 1). Sipuleucel-T was the first cancer vaccine to be approved by the FDA and the European Medicines Agency (EMA) as autologous cellular immunotherapy for the treatment of asymptomatic or minimally symptomatic, metastatic castrate-resistant prostate cancer [38]. Sipuleucel-T is thought to work through APCs to stimulate T-cell immune response targeted against prostatic acid phosphatase, an antigen that is highly expressed in most prostate cancer cells [39]. In castration-resistant prostate cancer, sipuleucel-T improved survival by 4 months [40].

Another type of cancer vaccine targets oncoviruses. During infection, some viruses insert their own DNA into host cells genome leading to malignant transformation of infected cells [41,42]. Cancer preventive vaccines mostly target cancer-causing viruses like human papilloma virus (HPV) or hepatitis B virus (HBV) and protect the host by stimulating the secretion of specific antibodies. HPV-vaccine Gardasil and several HBV-vaccines are two kinds of FDA-approved cancer preventive vaccines.

The Actual Place of Targeted Therapies on the Battlefield: The Good and Bad News

Until recently, chemotherapy or chemo-radiotherapy was often given as first-line treatment for advanced cancers. The emergence of targeted therapies was a real revolution since long-term complete tumour responses have been observed in different types of cancer, thus over performing the anti-tumour efficacy of standard of care usually given to patients [43,44]. These exciting results are shifting treatment goals in a proportion of patients with metastatic malignancy since higher responses rate and prolonged progression-free survival have become conceivable [43-45]. And some patients even undergo complete remission after targeted-therapy [46-49]. Consequently, the recommended guidelines for which drugs to use in which sequence dramatically changed. In metastatic melanoma and non-small cell lung cancer, anti-PD-1 agents (alone or in association with CTLA-4 blocking antibodies) and TKIs, like selective BRAF/MEK inhibitors, are now given in first-line treatment whereas chemotherapy takes the second place or is considered as a bridging treatment option. For CML, TKIs became the first choice with 85-95% of overall survival after 5 years. As for bevacizumab, an anti-VEGF antibody, it is largely administrated in combination with chemotherapy in colorectal cancer.

Despite important progresses, a large proportion of patients, depending on cancer types, still remain resistant to these targeted therapies and very few have shown complete remission. Furthermore, among patients who initially respond, a significant proportion undergo tumour relapse during the treatment, requiring patients to switch to one therapy to another with the hope to achieve cancer cell eradication [50]. But even in case of complete remission and despite regular follow-up, cancer recurrence can occur years after the end of the treatment [51,52], suggesting that undetectable residual tumour cells were unable to be eliminated and spread to other parts of the body. Avoiding the relapse by administration of preventive targeted-therapy may not be efficient, as illustrated by a study conducted in early-stage renal cell carcinoma (RCC) at high risk of recurrence [53]. Indeed, no difference of disease-free survival was observed between patients with resected local disease on anti-angiogenic drugs and

Table 2: Monoclonal antibodies approved by the FDA for the treatment of cancer.

Agent ANTICORPS	Target(s)	FDA-approved indication(s)
Alemtuzumab (Campath)	CD52	B-cell chronic lymphocytic leukemia
Atezolizumab (Tecentriq)	PD-L1	• Urothelial carcinoma • Non-small cell lung cancer
Avelumab (Bavencio)	PD-L1	Merkel cell carcinoma
Belimumab (Benlysta)	BAFF	Lupus erythematosus
Bevacizumab (Avastin)	VEGF ligand	• Cervical cancer • Colorectal cancer • Fallopian tube cancer • Glioblastoma • Non-small cell lung cancer • Ovarian cancer • Peritoneal cancer • Renal cell carcinoma
Blinatumomab (Blincyto)	CD19/CD3	Acute lymphoblastic leukemia (precursor B-cell)
Brentuximab vedotin (Adcetris)	CD30	• Hodgkin lymphoma • Anaplastic large cell lymphoma
Canakinumab (Ilaris)	IL-1β	• Juvenile idiopathic arthritis • Cryopyrin-associated periodic syndromes
Cetuximab (Erbitux)	EGFR (HER1/ERBB1)	• Colorectal cancer (*KRAS* wild type) • Squamous cell cancer of the head and neck
Daratumumab (Darzalex)	CD38	Multiple myeloma
Denosumab (Xgeva)	RANKL	Giant cell tumor of the bone
Dinutuximab (Unituxin)	B4GALNT1 (GD2)	Pediatric neuroblastoma
Durvalumab (Imfinzi)	PD-L1	Urothelial carcinoma
Elotuzumab (Empliciti)	SLAMF7 (CS1/CD319/CRACC)	Multiple myeloma
Ibritumomab tiuxetan (Zevalin)	CD20	Non-Hodgkin's lymphoma
Ipilimumab (Yervoy)	CTLA-4	Melanoma
Necitumumab (Portrazza)	EGFR (HER1/ERBB1)	Squamous non-small cell lung cancer
Nivolumab (Opdivo)	PD-1	• Colorectal cancer (dMMR and MSI-H) • Head and neck squamous cell carcinoma • Hodgkin lymphoma • Melanoma • Non-small cell lung cancer • Renal cell carcinoma • Urothelial carcinoma
Obinutuzumab (Gazyva)	CD20	• Chronic lymphocytic leukemia • Follicular lymphoma
Ofatumumab (Arzerra, HuMax-CD20)	CD20	Chronic lymphocytic leukemia
Olaratumab (Lartruvo)	PDGFRα	Soft tissue sarcoma
Panitumumab (Vectibix)	EGFR (HER1/ERBB1)	Colorectal cancer (*KRAS* wild type)
Pembrolizumab (Keytruda)	PD-1	• Classical Hodgkin lymphoma • Melanoma • Non-small cell lung cancer (PD-L1+) • Head and neck squamous cell carcinoma • Solid tumors (MSI-H)
Pertuzumab (Perjeta)	HER2 (ERBB2/neu)	Breast cancer (*HER2*+)
Ramucirumab (Cyramza)	VEGFR2	• Colorectal cancer • Gastric cancer or Gastroesophageal junction (GEJ) adenocarcinoma • Non-small cell lung cancer
Rituximab (Rituxan, Mabthera)	CD20	• Non-Hodgkin's lymphoma • Chronic lymphocytic leukemia • Rheumatoid arthritis • Granulomatosis with polyangiitis
Rituximab/hyaluronidase human (Rituxan Hycela)	CD20	• Chronic lymphocytic leukemia • Diffuse large B-cell lymphoma • Follicular lymphoma
Siltuximab (Sylvant)	IL-6	Multicentric Castleman's disease

Tocilizumab (Actemra)	IL-6R	• Rheumatoid arthritis • Juvenile idiopathic arthritis
Tofacitinib (Xeljanz)	JAK3	Rheumatoid arthritis
Tositumomab (Bexxar)	CD20	Non-Hodgkin's lymphoma
Trastuzumab (Herceptin)	HER2 (ERBB2/neu)	• Breast cancer (*HER2+*) • Gastric cancer (*HER2+*)

those that received placebo [50]. A possible explanation might rely on the poor vascularization of early-stage tumour site hinders the access of systemic cancer therapy in the tumour microenvironment. In addition, to the difficulty of destroying cancer cells, the toxicities of targeted-therapy include various symptoms like cutaneous and gastrointestinal toxicity, B cell aplasia, CRS or neurotoxicity [54-56]. Although reversible in most instances, these toxicities require specific medical interventions [32].

Overall, all these targeted strategies and their outcomes risen two important points. First, using one drug to target one pathway is not enough to win the war against cancer. Second, tumours have the capacity to evolve and adapt in response to external attacks. This raises the following questions: is it possible to adapt cancer targeted-therapy according to tumours evolution and how? Could we identify specific biomarkers to predict what patients are likely to benefit from target-therapy while reducing immune-related adverse events? Further understanding of the genetic and epigenetic alterations that take place in cancer cells and causes treatment failure may be a first step toward the development of better-adapted therapeutic strategies throughout the course of the disease.

Genetic Diversity and Epigenetic Plasticity are Key Source of Information to Fight Cancer

One reason explaining why it is so difficult to fight against cancer is that tumours harbour a striking heterogeneity and this intra-tumour heterogeneity evolves during the disease course [57-59]. Thus, a precious source of information to develop cancer treatments lies in our understanding of this heterogeneity, its origins and underlying mechanism. Stem cells have a central role in the clonal evolution of cancer cells leading to tumour heterogeneity [60-63]. Normal stem cells are prime targets for the initiation of malignant transformation [64] but downstream progenitors, prior to terminal differentiation, can also acquire self-renewal capacity by mutational changes [65] or micro-environmental pressures, as in zones of hypoxia [66] or with metastatic spread and epithelial-mesenchymal phenotypic transition [67]. As a consequence, cancer stem cell populations are genetically diverse in individual patients [68-71] (Figure 2). After cancer initiation, multiple sub-clones often co-exist with no clear fitness advantage [71-73]. Within tissue microenvironments, cancer sub-clones indulge in reciprocal dialogues with each other and with stromal, endothelial and immune cells, modulating each other in the struggle to maximise fitness [74-76]. This is clearly illustrated by the concept of cancer immunoediting in which while protecting the host against tumour cell spreading, the immune system indeed shapes the tumour by editing its genome and giving birth to novel tumour subclones [77,78].

Another key source of information for precision therapies development comes from a deeper understanding of genetic and epigenetic tumour features. Indeed, malignant transformation, oncogenesis and tumour growth are governed by mutations and epigenetic changes [79,80]. Oncogenes activation (*c-MYC, WNT1, HER2, KRAS*…) and tumour suppressor genes silencing (*TP53, CD95*…) are important factors that can be regulated during these processes [81,82]. In addition, epigenetic aberrations or inactivation of genes responsible for protecting DNA integrity are able to support highly mutable phenotypes [83-86]. For example, hypomethylation near guanine quadruplexes increases the rate of DNA breakage and activation of homologous recombination may also act as a mutagenic factor [87].

During carcinogenesis the accumulations of tumour genomic alterations influence the response to therapy. For example, in colorectal cancer (CRC), tumours are classified according to their somatic mutation profiles. A deficiency in DNA mismatch repair system is reflected by a microsatellite instable status (MSI), which is associated with treatment outcome. Notably, CRC with MSI were unexpectedly responsive to immune checkpoint therapy targeting PD-1/PD-L1 pathway [88]. This observation is consistent with the specific enrichment of mutations in DNA repair gene *BRCA*2 in

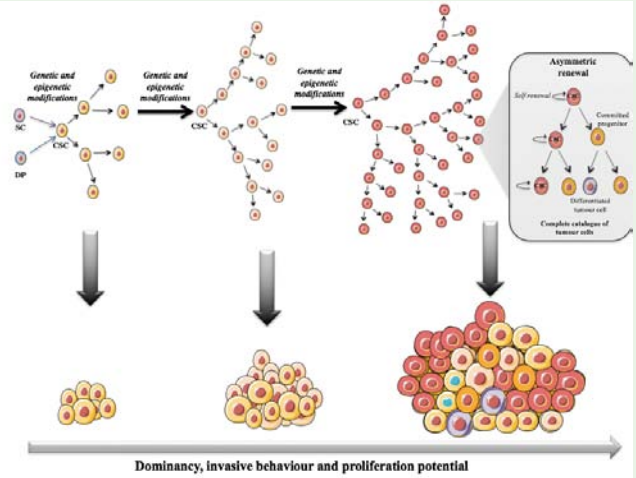

Figure 2: Clonal evolution model of cancer stem cells in the establishment of heterogenous tumors.

In the illustrated model Cancer Stem Cells (CSCs) could arise through mutations acquired in Stem Cells (SCs) or could also originate from differentiated or progenitor cells that have regained 'stemness', a term used to refer to the intrinsic molecular pathways, epigenetic modifications and particular transcription factors that regulate and maintain the SC form [148]. An ancestral CSC could give rise to one or two separate clonal lineages that independently evolve. More precisely, the acquisition of genetic mutations could produce complex genetically diverse branches of CSCs that vary in dominancy and malignancy. CSCs can divide asymmetrically, giving rise to one daughter CSC and one committed progenitor tumour cell, which has limited proliferative capacity. This leads to generation of the complete catalogue of tumour-comprising cells [148].

metastatic melanoma responding to anti-PD-1 therapy [89]. By contrast, tumours overexpressing genes involved in mesenchymal transition, cell adhesion, angiogenesis or wound healing were naturally resistant to this treatment [89]. Mutations arising in genes like FLT3, DNMT3A or splicing factors predict poor prognosis and are associated with chemotherapy resistance in hematopoietic malignancies, especially for patients with tumour recurrence [90-93]. It was reported that gene silencing through promoter hypermethylations in tumour-associated genes can reduce patient's survival by disrupting the response to chemotherapy [94-96].

In his seminal 1976 review, Nowell described cancer clone development as mechanism of diversification and selection in the context of tissue ecosystem pressures [97-101]. In such context cancer therapeutics is one the most potent ecosystem selection pressure in cancer [97-101]. Importantly, cancer therapy can drive the selection of resistant subclones [97]. This is clearly evident with TKI resistance, which can be allocated to somatic mutations in the targeted genes able to drive their reactivation and disable TKI action [102]. Similarly, when hematopoietic cancers are predated by allogenic transferred T cells, genomic deletion of mismatched HLA alleles selects for immunological invisibility [103,104]. Interestingly, therapeutic escape also relies on epigenetic routes to deregulate the expression of the targeted genes or other pathways that will interfere with treatment efficacy [105,106].

In accordance to aforementioned mechanisms underlying tumours origins and development, molecular strategies need to incorporate an evolutionary view of malignant transformation modulated by networks of genetic and epigenetic interactions to provide effective treatment across cancer subtypes.

Evolutive Tumour Profiling: A Path toward Evolutionary Therapies

Following the idea that cancer evolution is fuel by mutations to converge towards metastasis and drug resistance phenotypes [107,108] we can explore novel evolutionary approaches to therapy. For example, in advanced melanoma and lung cancer, high levels of somatic mutations are associated with improved clinical outcome after immune checkpoint blockade therapy [109,110]. Importantly, innate or acquired somatic mutations can alter wild-type proteins and create mutated neo-epitopes potentially targetable by T cells [111,112]. Eliciting a broad and evolving response to tumours appear then as an appealing strategy, opening the way to neo-epitopes-based T cell therapies such as adoptive T cell transfer or vaccines [112]. Neo-epitopes identification for targeted cancer immunotherapy starts with exome and RNA sequencing of cancer and matched normal cells to detect mutated sequences. Then data are processed in computational pipelines for epitope prediction. Finally, selected neo-peptides are synthetized and selected for their capacity to be recognised by specific T cells [113-115]. To avoid any cross-reactivity of T cell against native antigens, targeted neo-epitopes should ideally derive from antigens specifically expressed by tumour cells such as WT1, *HER2*/Neu or the telomerase reverse transcriptase subunit (TERT).

A large fraction of mutations in cancer cells arise from a stochastic process and are not shared between patients, making them patients specific. In this condition targeting neo-epitopes for would require a personalized therapy [116,117]. Fortunately, although mutational load in cancer is heterogeneous, not all somatic mutations randomly occur. Cancer types are also associated with shared mutation load, giving rise to common newly created epitopes referred as "public" neo-epitopes [111,118,119]. Indeed, mutations that promote oncogenesis can systematically appear across patients [118]. An example concerns telomerase antigen, which could particularly be an interesting target. Telomerase activity is required to maintain cancer cell immortality [120,121] and all mutations described in TERT promoter led to its over-activation [122,123]. Hence, due to its critical property in oncogenesis, tumour escape by TERT antigen loss mechanism is clearly reduced [124]. The sharp rise of telomerase expression following TERT promoter mutation in cancer cells could eventually reveal previously undetectable epitopes that may thus be considered as tumour neoepitopes to target for immunotherapy.

Currently, combination regimens are key strategies to treat advanced-stage disease with the goal to reverse acquired resistance [125]. The development of secondary mutations, gene amplifications, and late activation of signal-transduction pathways in tumour cells are common in the development of acquired resistance [126]. Adding a second drug as part of a combination regimen in this setting takes the dynamic nature of clonal evolution into consideration, and assumes that the tumour consists of clones that remain sensitive to the first drug and that addition of the second drug to the therapy combination will target clones resistant to the first drug. An example of this type of combination therapy involves the association of *HER2*-targeting drugs with mTOR inhibitors in *HER2*-positive advanced-stage breast cancer [127-129], in which secondary mutations in *PIK3CA* or increased signalling though PI3K have been shown to are mechanisms of acquired resistance to *HER2* inhibition [130].

Aside from bsAb previously discussed, another interesting approach for anticancer combinatorial therapy is the recent development of bifunctional molecules, which consists in antibody-cytokine fusion proteins named "immunocytokines". The goal of this approach is to directly bring the cytokine into the tumour. It has been reported that TGFβ signalling confers resistance to anti-PD-1/PD-L1 therapy limiting the treatment efficacy [89]. The lack of response to anti-PD-L1/PD-1 therapy was associated with TGFβ, especially for tumours with an immune–excluded phenotype [89,131]. The bifunctional protein M7824, combine an anti-PD-L1 antibody linked to the extracellular domain of TGFβ receptor 2 TGFβR2 and acts as a TGFβ Trap. Preclinical studies in mice revealed that M7824 reverse the immune–excluded phenotype by fostering T cell localization to the tumour bed. Preliminary results from a phase 1 trial of M7824 indicate that this therapy is well tolerated and 2 phase I trials are currently ongoing in patients with advanced solid tumours (NCT02517398, NCT02699515).

In the future, innovative approaches might involve adding the second drug when resistance has occurred following an initial response to the first drug. To do so, a key question remains to design new strategies against cancer: can we predict tumour evolution before it happens? Accurately measuring and modelling intra-tumoral genetic and epigenetic heterogeneity would help to determine biomarkers that indicate if therapy is successful during the course of treatment or when a resistance appears. To predict genomic changes during treatment, tumour biopsies should ideally be performed regularly to monitor for cues to initiate a combination before

resistance occurs. However, such invasive process is obviously not conceivable. A more realistic approach would be greatly facilitated by the analysis of circulating tumour DNA (ctDNA) [132-134]. In one study of melanoma, ctDNA was found to be relatively consistent and informative as a blood-based biomarker [135]. Levels of ctDNA corresponded to response and disease progression. Similarly, a study in breast cancer found that ctDNA predicted metastatic relapse for patients with early-stage disease and was able to predict they genetic events found in the metastatic relapse [136]. Beyond predicting relapse, ctDNA may also offer insight into mechanisms of resistance. For example, RAS pathway mutations have been detected by ctDNA as a mechanism of resistance in colorectal cancer to anti-EGFR therapies [137-139]. Measuring epigenetic alterations in ctDNA is also possible. Indeed, numerous methylated biomarkers have been established to correlate with disease progression [140-144]. Our new understanding of cancer as a phenotype influenced by gene expression and modulated by epigenetic factors is currently guiding the development and selection of targeted therapies. In some cancers, a molecular disease classification is routinely performed at the diagnosis to know if a specific targeted therapy can be preferentially applied in first-line [145-147]. But only few parameters are investigated and more robust molecular/genomic analysis is still required to better characterize cancer evaluative features and treat patients accordingly.

Conclusion

Our continuous increasing understanding of cancer biology has led to the development of molecularly targeted anticancer therapies that considerably increased the survival of cancer patients. However, the initial euphoria of early breakthroughs exploiting targeted treatments was followed by disappointment related to the observation of resistance to large numbers of these agents and, later, acquired resistance in patients who had an initial response. As a consequence, the thinking surrounding the development of anticancer strategies is evolving. Cancer is an evolutionary process in which genetics and epigenetics intertwined at every step given rise to a striking intra-clonal genetic and epigenetic diversity. As a consequence, we have to master tumour heterogeneity to achieve optimal combinatorial deigns of targeted therapies. Precise biomarkers need to be developed to monitor precision therapy and subclonal dynamic of tumour architecture. Although we still have to face considerable challenges, there is much to celebrate in the advancing of cancer treatment. Newer technologies to widespread our ability to serially profile genomic, transcriptomic, and epigenetic events in cancer cells, are allowing to fine-tune therapeutic approaches to improve patient scare.

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