My journey from tyrosine phosphorylation inhibitors to targeted immune therapy as strategies to combat cancer

Alexander Levitzki[a,1] and Shoshana Klein[a]

[a]Unit of Cellular Signaling, Department of Biological Chemistry, Silberman Life Sciences Institute, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected in 2017.

Contributed by Alexander Levitzki, February 6, 2019 (sent for review September 24, 2018; reviewed by Joseph Schlessinger and Nahum Sonenberg)

Since the 1980s there has been a drive toward personalized targeted therapy for cancer. "Targeted cancer therapy" originally focused on inhibiting essential tumor survival factors, primarily protein tyrosine kinases. The complexity and rapid mutability of tumors, however, enable them to develop resistance to tyrosine kinase inhibitors (TKIs), even when these are multitargeted or applied in combination. This has led to the development of targeted cancer immunotherapy, to enhance immune surveillance against the tumor. In this paper, we provide a personal view of the development of targeted therapy, from TKIs to targeted immunotherapy.

targeted therapy | tyrosine kinase | immunotherapy | cancer | tyrphostin

Until the 1980s the fight against cancer focused mainly on surgery, chemotherapy, and radiotherapy. The discovery of oncoproteins and their central roles in the initiation, propagation, and metastasis of cancer moved the fields of clinical and basic cancer research in new directions. The identification of pp60Src as a protein tyrosine kinase (PTK) (1, 2) and the identification of many other PTKs as key cancer-driving proteins (3) propelled the field toward a molecular understanding of oncogenesis. This in turn led to the development of "targeted therapies," which inhibit the key molecules involved in tumor initiation and progression (Fig. 1).

The idea of treating cancer by targeting proteins that play pivotal roles in the disease was actually validated decades before PTKs took center stage. As early as 1941, it was shown that metastatic prostate cancer could be treated by androgen deprivation, by means of castration or estrogen injection (4). Androgen deprivation therapy remains a mainstay of prostate cancer treatment to this day. Tamoxifen, a partial agonist of the estrogen receptor, was introduced in the 1970s for the treatment of advanced breast cancer (5). Tamoxifen, as well as the newer aromatase inhibitors, is routinely used today to treat hormone-positive breast cancer and to prevent breast cancer in women at high risk of developing new or recurrent disease. Hormone-targeted agents such as tamoxifen, with their focused action and consequent diminished toxicity, provided the proof of principle for targeted cancer therapy.

Tyrosine Phosphorylation Inhibitors

The emergence of PTKs as essential elements in the initiation, progression, and metastasis of cancer drew us into the field of cancer therapy. My (A.L.) earlier experience in combining chemistry with biology in the study of signal transduction (6, 7) convinced me that it was possible to synthesize specific inhibitors of tyrosine kinases. Many in the scientific community thought this goal was unattainable—the NIH denied my grant proposal—on the grounds that the extensive homology between the ATP-binding domains of tyrosine kinases precluded selectivity. Despite the skeptics, my laboratory pioneered the systematic synthesis of tyrosine phosphorylation inhibitors, or "tyrphostins," commonly known as tyrosine kinase inhibitors (TKIs).

In the early 1980s the only enzymatically active PTKs that had been purified were the insulin receptor kinase (InsR) and the epidermal growth factor receptor kinase (EGFR). Using these receptor kinases, we established a platform to generate and test for inhibitors of their tyrosine kinase activities. Most importantly, we looked for inhibitors that could discriminate between InsR and EGFR. At that time, we did not attempt to target the more ubiquitous serine/threonine kinases. These are four times more abundant than PTKs and are involved in numerous biochemical pathways, so we worried that serine/threonine kinase inhibitors would have serious side effects. PTKs are less abundant than serine/threonine kinases and more focused on signal transduction. Their aberrant activity in various cancers had already been documented when we entered the field.

In 1983, my graduate student, Pnina Yaish, in collaboration with my colleague Chaim Gilon, embarked on a project to design and synthesize inhibitors of the InsR and EGFR kinases. Shortly thereafter Aviv Gazit joined the laboratory as a postdoctoral fellow, later becoming a research associate. Patterning our compounds around a variety of aromatic nuclei (8–10), we developed a series of tyrphostins that could discriminate between the InsR and EGFR kinases. We had compounds with differences of orders of magnitude in their affinities for the two receptors, and a plausible structure–activity relationship (11). As we had anticipated, the EGFR kinase inhibitors also inhibited the EGF-dependent proliferation of EGFR-overexpressing tumor cells (8, 11).

Our discoveries heralded a new approach to the treatment of cancer: the inhibition of oncogenic PTKs. Moving ahead on a broad front, within a few years we were able to synthesize Bcr-Abl

Significance

This paper discusses the successes and failures of tyrosine kinase inhibitors in treating cancer, and why we believe targeted immunotherapy may provide more durable remissions. Tyrosine kinases are central to tumor development and progression. Since we originally demonstrated specific inhibition of tyrosine kinases, with minimal toxicity, a large number of tyrosine kinase inhibitors—both small molecules and antibodies—have entered the clinic. Nonetheless, tumors are heterogeneous and highly mutable, and they eventually bypass tyrosine kinase inhibition. The immune system has the ability to seek and destroy tumor cells. Tumors avoid detection by the immune system, so the goal is to reinstate immune surveillance against the tumor.

Author contributions: A.L. and S.K. wrote the paper.

Reviewers: J.S., Yale School of Medicine; and N.S., McGill University.

Conflict of interest statement: A.L. is a founding board member of TargImmune Therapeutics Inc, which is developing the targeted polyIC strategy for the clinic.

See QnAs on page 11562.

[1]To whom correspondence should be addressed. Email: alex.levitzki@mail.huji.ac.il.

Published online May 10, 2019.

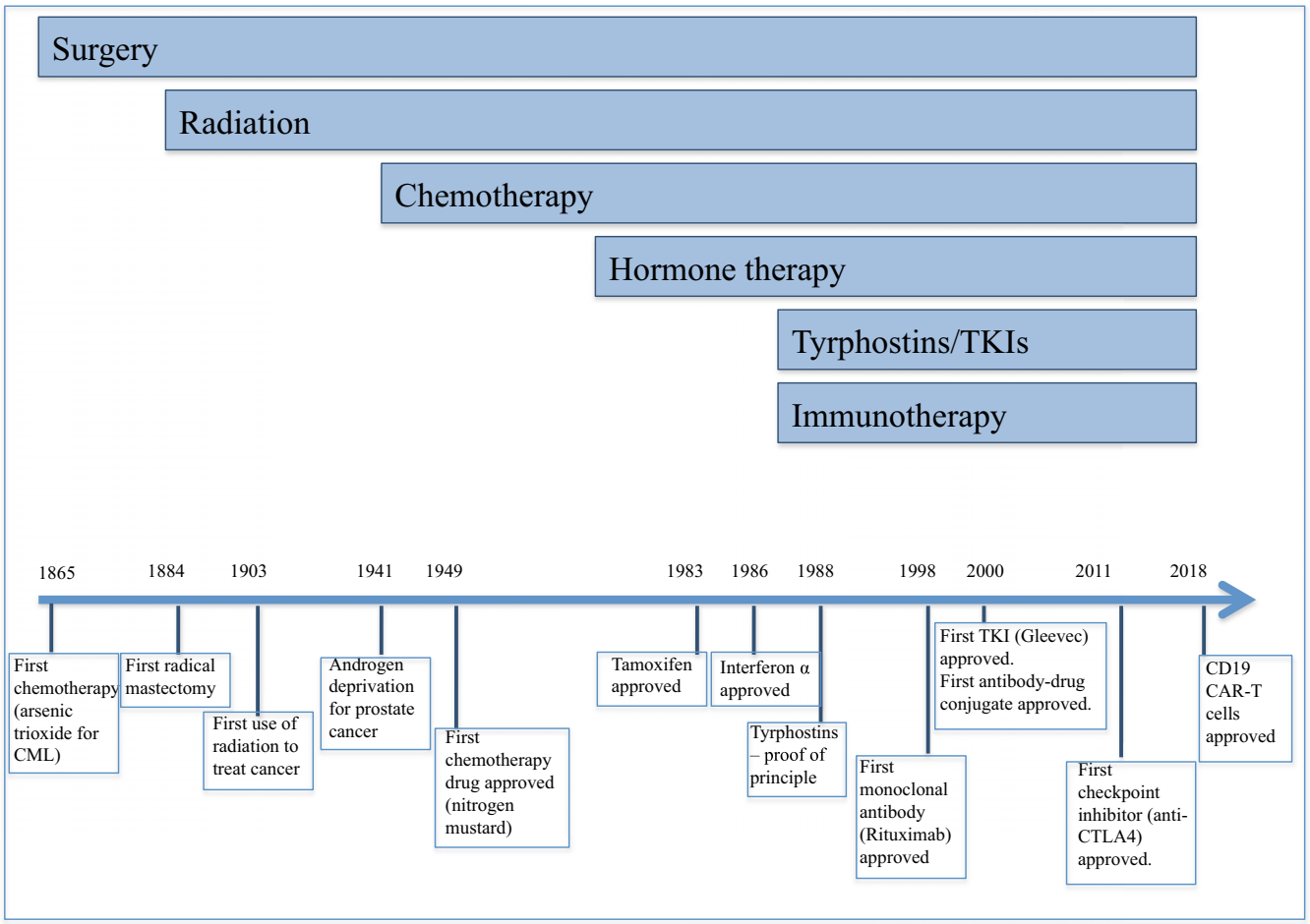

Fig. 1. The growing arsenal of anticancer therapeutics. Effective control of cancer requires appropriate combinations. Immunotherapy has the potential to hunt out distant metastases and provide long-term solutions.

inhibitors, and to show that these killed K562 chronic myelogenous leukemia (CML) cells (12, 13). In parallel we generated highly selective inhibitors of platelet-derived growth factor receptor (PDGFR) (14, 15), vascular endothelial growth factor receptor (VEGFR) (16), Janus kinase 2 (JAK-2) (17), and insulin-like growth factor 1 receptor (IGF1R) (18, 19). The stage was set for the clinical development of these and similar PTK inhibitor molecules (reviewed in ref. 20).

Brian Druker and Nick Lydon of Novartis took up the challenge of developing Bcr-Abl inhibitors as cancer drugs (21, 22). The result was imatinib (Gleevec), the first TKI to be approved for the clinic. Gleevec dramatically changed the fate of patients diagnosed with CML at the early, chronic stage. Chronic CML cells are initially dependent for their survival on Bcr-Abl kinase, generated by a reciprocal translocation between the *BCR* and *ABL1* genes [t(9;22)]. By inhibiting the activity of Bcr-Abl, Gleevec destroys early CML cells. Chronic CML used to come with a grim prognosis. Today, Gleevec allows patients who are diagnosed early enough to live for many years.

At the later, acute stage of CML, additional "drivers" come into play, and Gleevec is effective for much shorter periods (23). CML at the late phase is like the majority of tumors, which are driven by a multitude of aberrant signaling pathways rather than depending on a single oncogene. Thus, alternate survival pathways quickly bypass a single targeted drug. In this sense, the success of Gleevec in curing early-stage CML is an "outlier." However, it provided the impetus for the development of many other targeted kinase inhibitors, which lie at the core of cancer therapy today.

Combination Therapies

The success of Gleevec in the treatment of early CML led us and others to hope (rather naively) that our pioneering work on EGFR and Bcr-Abl kinase inhibitors, would set the foundation for the cure of other cancers (24, 25). The first sobering experience was the failure of two potent EGFR kinase inhibitors, gefitinib (Iressa) and erlotinib (Tarceva), in clinical trials for the treatment of lung cancer. EGFR had been considered a prime target for cancer therapy, as it is overexpressed in a wide variety of tumor types, including lung cancer. However, only about 10% of lung cancer patients responded to Iressa or Tarceva, and in most of those the response was short-lived. Later it became apparent that the tumors that were affected by these inhibitors had specific activating mutations in the EGFR, which caused them to be "addicted" to the mutated receptor (26). In the absence of these mutations, tumor survival does not appear to depend on EGFR activity and is refractory to EGFR inhibition, even in cases where the EGFR is highly overexpressed on the tumor.

In those patients whose tumors initially respond to EGFR inhibition, the disease almost always recurs. Tumors mutate rapidly, so initial drug responsiveness is followed by the appearance and outgrowth of resistant tumor cells. Sometimes, resistance mutations preexist in a subset of cells of the tumor before therapy, and the therapy serves to select for the resistant cancer cells. Treating cancer with tyrphostins has come to resemble chasing a runaway cart, as second- and third-generation

compounds are developed to contend with the emergent mutations. Thus, the Bcr-Abl inhibitor imatinib gave rise to nilotinib, a more selective and more potent derivative that also inhibits some of the kinase point mutants, and these have been joined by the structurally unrelated compounds dasatinib and bosutinib (27). Similarly, the first-generation EGFR inhibitors, gefitinib and erlotinib, were followed by the second-generation afatinib and dacomitinib, and the third-generation osimertinib. Patients who initially respond to gefitinib eventually develop resistance mutations that can be combated by osimertinib as a second line of care, but it is only a matter of time until the tumor becomes resistant to osimertinib as well (28, 29).

At first we strove to develop extremely selective inhibitors, believing that these would have minimal side effects. Nonetheless, since tumors are usually heterogeneous and have multiple drivers (early CML is the exception, rather than the rule), we believed it might be necessary to inhibit multiple pathways. We therefore anticipated that these drugs would be most potent in combinations (25). The current attempt of the cancer community is to combine targeted agents with one another, or with cytotoxic drugs. These combinations frequently prolong disease-free survival, but they rarely improve overall survival or provide a long-term cure (30, 31). One cannot compare cancer to AIDS or infectious diseases, in which combinations have long-lasting effects. The networks that drive cancer are extremely complex and elaborate. Furthermore, cancer networks evolve continuously, and even more so during treatment. Another compounding issue is that the network of the primary tumor is often very different from that of the metastatic lesions, even though single-cell analyses indicate metastases can evolve from subclones within the primary tumor (32–34). Thus, this type of combinatorial therapy is unlikely to provide a complete cure.

Multitargeted Tyrphostins

Nevertheless, some of the most successful TKIs have been "multitargeted" drugs, which hit more than one driver (35). Indeed, Gleevec inhibits not only Bcr-Abl but also both the PDGFR and KIT kinases and is therefore approved for the treatment not only of CML but also of gastrointestinal tumors that depend on these receptors (36).

It is not easy to screen for multiple targets, and it is very difficult to optimize multiple targeting by a single drug. The finding that a compound homes to a number of targets is usually serendipitous. In recent years, our own laboratory's quest for improved IGF1R inhibitors led to the development of the NT family of tyrphostins, including NT157 and NT219. While these strongly inhibit the IGF1R/insulin receptor substrate 1/2 (IRS1/2) pathway (37), it turned out that they also induce the dephosphorylation of tyrosine residue 705 of signal transducer and activator of transcription (STAT3) (38). The NT family hits yet other targets, as we found with our colleague Roger Daly (39). Both IRS1/2 and STAT3 act as tumor drivers and at the same time affect the tumor microenvironment (TME).

The transcription factor STAT3 is a prime target for cancer therapy. STAT3 directly stimulates tumor growth and survival, as well as promoting a TME that is conducive to tumor growth and survival. Activation of STAT3 within the tumor induces the formation of cytokines and chemokines, which activate STAT3 in immune cells. STAT3 represses cytotoxic T cells, natural killer (NK) cells, and neutrophils and inhibits dendritic cell maturation, leading to suppression of the antitumor immune response (40, 41). It is therefore not surprising that inhibitors of STAT3 and its upstream activators have been pursued for over two decades. Our finding that NT157 inactivates STAT3 by dephosphorylating PY(705) provided the impetus for us to search for small molecular compounds that dephosphorylate PY(705)STAT3 in whole-cell assays. In addition to trying to unravel the mechanism of STAT3 dephosphorylation by NT157, we aim to find additional small molecules that inhibit STAT3 by dephosphorylation.

Until NT157, all STAT3 inhibitors targeted either the dimerization of phosphorylated STAT3 or the binding of the activated STAT3 dimer to DNA. None of these inhibitors achieved clinical approval, mainly because of toxicity issues (42). NT157, unlike other STAT3 inhibitors, had very low toxicity in preclinical studies. Indeed, our experience has been that tyrphostins tend not to be very toxic. Michael Karin's group tested NT157 on the spontaneous development of colon cancer in CPC-APC mice, which lack the *APC* tumor suppressor. NT157 reduced tumor burden and led to the production of multiple cytokines and chemokines by the TME. NT157 decreased the migratory activity of cancer cells in vitro and in vivo (37, 43). Hence, NT157, which inhibits the signaling of both the IGF1R/IRS and STAT3 pathways, affects both the tumor and its microenvironment and is effective against a broad range of cancer cell types. These findings establish a new paradigm, namely, that one should seek multitargeted inhibitors or nontoxic combinations of drugs that affect both the tumor and its TME.

Targeting the Immune System to Tumors

The realization that cancer is an ever-evolving moving target has led to attempts to harness the immune system against the disease. It has long been recognized that the immune system has the potential to hunt and destroy cancer cells (44–46). The first efforts to harness the immune system to combat cancer involved designer antibodies targeted against antigens that are overexpressed on cancer cells. Rituximab (Rituxan), the first monoclonal antibody to be approved by the Food and Drug Administration, targets CD20 and is used in the treatment of B cell leukemias and lymphomas. This was followed by trastuzumab (Herceptin), which blocks the activation and signaling of HER-2, an EGFR family member that is frequently overexpressed in breast and other cancers. These antibodies are most effective when they not only block their targets but also induce Fc-mediated antibody-dependent cytotoxicity (47). These successful antibodies paved the way for many more antibodies to enter the clinic, for cancer and other indications. Examples include cetuximab (Erbitux), which targets the EGFR, and is approved for colorectal and head and neck cancers, and bevacizumab (Avastin), which targets VEGF to inhibit angiogenesis. In addition to its use as an anticancer drug, mostly for colon cancer, bevacizumab prevents retinal damage caused by age-related macular degeneration.

Antibodies can also be used to deliver therapies to specific sites. Antibody–drug conjugates (ADCs) have been developed to target cytotoxic drugs to tumors, minimizing side effects. The first ADC to be approved was gemtuzumab ozogamicin, for acute mylogenous leukemia. Trastuzumab emtansine (T-DM1), for the treatment of HER-2–positive metastatic breast cancer, is the only ADC currently approved for a solid tumor. Many other ADCs are in clinical trials (48, 49).

In parallel with the development of therapeutic monoclonal antibodies, efforts are underway to enhance T cell-mediated effects. In one approach currently in clinical trials, dendritic cells are loaded ex vivo with tumor antigens, to induce T cells to respond to tumors carrying these antigens (50–52). In another approach, known as adoptive T cell therapy, tumor-infiltrating lymphocytes are removed and expanded ex vivo and returned to the patient. Recent success has highlighted CAR-T cells, which are engineered ex vivo to express a chimeric antigen receptor and are then infused back into the patient (53). CAR-T cells designed to target CD19 can lead to complete remission (54, 55) and were recently approved for the treatment of pre-B cell acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma. Much effort is now being directed to expanding the use of CAR-T cells to other cancers, including solid tumors, and to developing universal, off-the-shelf versions (56–58).

Another approach to improving T cell-mediated anticancer immunity is to remove immune checkpoints. Several antibodies

that target immune checkpoints have been approved, including anti–CTLA-4 (ipilimumab), anti-programmed cell death-1 (PD-1) (nivolumab and pembrolizumab), and anti-programmed cell death ligand-1 (PD-L1) (atezolizumab and durvalumab), and they are now the standard of care for certain cancer types, including metastatic melanoma, non-small-cell lung cancer, renal cancer, and Hodgkin's lymphoma. Some patients are intrinsically resistant to this type of therapy, whereas others respond to the therapy after a delay. Moreover, there have been several reports of patients whose tumors actually progressed more rapidly after treatment with checkpoint inhibitors (59, 60), and it has been suggested that PD-1 can act as a tumor suppressor in some contexts (61).

Like other cancer therapies, a positive initial response to current immunotherapies is sometimes followed by the development of resistance. The mechanisms of innate and acquired resistance to immune checkpoint inhibition are under intense study (62–64). Tumors sporting a large repertoire of neoantigens, such as colon cancers with defective mismatch repair and microsatellite instability, are both more immunogenic and more responsive to checkpoint blockade than tumors with few tumor-specific antigens (65–67). Accordingly, loss of tumor antigens dampens the antitumor immune attack. In a study of ALL patients who initially responded well to CAR-T cells directed against CD19 and later relapsed, a majority had lost the CD19 marker on their B ALL cells, rendering them resistant to the therapy (68). The take-home message from this study is that one should target more than a single antigen.

Like antigen loss, impaired antigen processing due to lack of MHC components also minimizes the response to immunotherapy (69–71). Further, defects in T cell maturation and/or tumor infiltration can lead to resistance. For example, loss of phosphatase and tensin homolog (PTEN) has long been known to activate the PI3K/Akt pathway, leading to enhanced tumor proliferation and resistance to apoptosis. In mice, PTEN loss also leads to reduced T cell infiltration into the tumor, so the tumor becomes refractory to anti–PD-1 therapy (72). These mechanisms of resistance were highlighted in a recently reported case study: A sarcoma patient who responded well to anti–PD-1 therapy had a single resistant metastasis (fortunately, resectable). The resistant tumor had lost PTEN and also had reduced expression of two T cell reactive tumor neoantigens (73). Studies of this nature provide clues as to useful combinations between kinase inhibitors (e.g., PI3K and Akt) and checkpoint blockade.

The TME, including the immune cells in the neighborhood, also plays a role in the response to immunotherapy. Alternative checkpoints can stymie a particular checkpoint therapy. For example, TIM-3 is an inhibitory receptor, expressed on CD4+ T helper cells and CD8+ cytotoxic T cells (74). The combination of anti–TIM-3 and anti–PD-1 was effective in preclinical models (75–77). Moreover, two lung cancer patients who relapsed following anti–PD-1 treatment had increased TIM-3 on their T cells (78), suggesting that their tumors escaped the PD-1 blockade by increasing the strength of the TIM-3 checkpoint. In an intriguing preclinical study, tumor-associated macrophages were seen to capture anti–PD-1 antibodies from the surface of the T cells to which they had attached. This effect was abrogated by blocking the Fcγ receptors, suggesting a possible avenue for improving the response to anti–PD-1 (79).

Although most patients eventually relapse after immunotherapy, a subset of patients experience long-term complete remission (80). In these patients, an immunological memory prevents regrowth of the tumor. In light of this success, many more checkpoint inhibitors are in the pipeline. In attempts to understand which patients are most likely to respond in a positive fashion, and what features lead to long-term remission, several studies have defined molecular signatures associated with resistance to checkpoint blockade (81–83). These studies will help in developing strategies to maximize the response.

An exciting development is the emerging utilization of cancer-homing oncolytic viruses (84). These viruses induce the lysis of cancer cells, leading to the release of numerous cancer antigens, stimulating an anticancer immune response. T-VEC (talimogene laherparepvec) was approved in 2015 for treating advanced melanoma. In a recently reported phase I–II clinical trial, 20% of glioblastoma patients who received a recombinant oncolytic poliovirus were alive after 3 y, which is much longer than the usual prognosis (84). If these results are maintained in phase III trials, they will offer hope for patients with this deadly tumor.

In addition to these strategies, one should not forget the good old cytokines, IL-2 and IFN-α, which still occupy a safe place in the anticancer pharmacopeia, especially in combination with other therapies. IFN-α is used in the treatment of hairy-cell leukemia, AIDS-related Kaposi's sarcoma, follicular lymphoma, CML, and malignant melanoma (85–88). IL-2 is used in the treatment of malignant melanoma and renal cell carcinoma (89, 90). Combining checkpoint immunotherapies with pharmaceutical agents is being investigated intensively. Immunostimulatory drugs such as colony stimulating factor 1 receptor inhibitors and Toll-like receptor (TLR) agonists have been particularly effective in this setting (91–95).

Targeting Polyinosinic/Polycytidylic Acid to Tumors

Recognizing the promise of immune therapy, we moved the focus of our laboratory toward targeted immunotherapy. Our first foray into immune therapy involved the cytokine-activated JAK/STAT pathway. Long ago, we showed with Chaim Roifman that ALL was characterized by JAK-2 activation and was inhibited by the tyrphostin AG-490 (17). Together with Richard Jove, we then demonstrated that AG-490 was synergistic with IL-12 immunotherapy in STAT3-dependent murine myeloma (96). Although IL-12 was more or less abandoned as a therapeutic strategy, owing to toxicity, there has recently been a reawakening of interest, and several groups are working on targeting its delivery, to minimize toxicity (97, 98).

Over the past decade and a half, we have developed strategies to target polyinosinic/polycytidylic acid (polyIC), a form of synthetic long-chain dsRNA, to tumors. PolyIC has long been known to be potent against tumors, but systemic delivery causes unacceptable toxicity. We have constructed both chemical and protein-based vectors that bind and carry polyIC to cancer cells that overexpress a membrane-bound receptor, such as the EGFR (Fig. 2) (99, 100). PolyIC mimics viral dsRNA to provoke a profound antiviral attack. The major receptors for dsRNA include dsRNA-dependent protein kinase (PKR), TLR3, melanoma differentiation-associated protein 5 (MDA5), and retinoic acid-inducibe gene I (RIG-I) (101); their activation induces the secretion of type I IFNs, leading to expression of IFN-stimulated genes, immune modulation, and apoptosis (Fig. 3) (102–105).

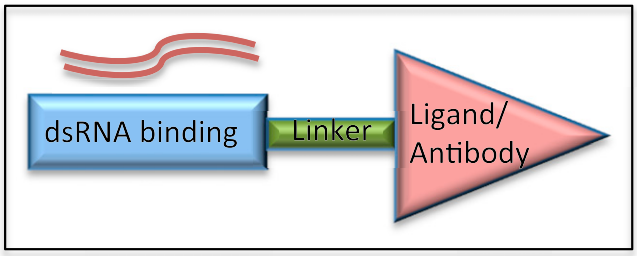

Fig. 2. Vectors that carry PolyIC, a synthetic dsRNA. The vectors we designed are built from three parts: (*i*) A polyIC binding moiety, such as polyethyleneimine (PEI). PEI also functions as a "proton sponge," causing the endosome to swell and burst, releasing the dsRNA into the cytoplasm, where it interacts with its molecular targets. (*ii*) A linker, such as PEG. (*iii*) A homing ligand or antibody, which guides the vector to the appropriate cells. The ligand is aimed at a receptor that is overexpressed on the cancer cell.

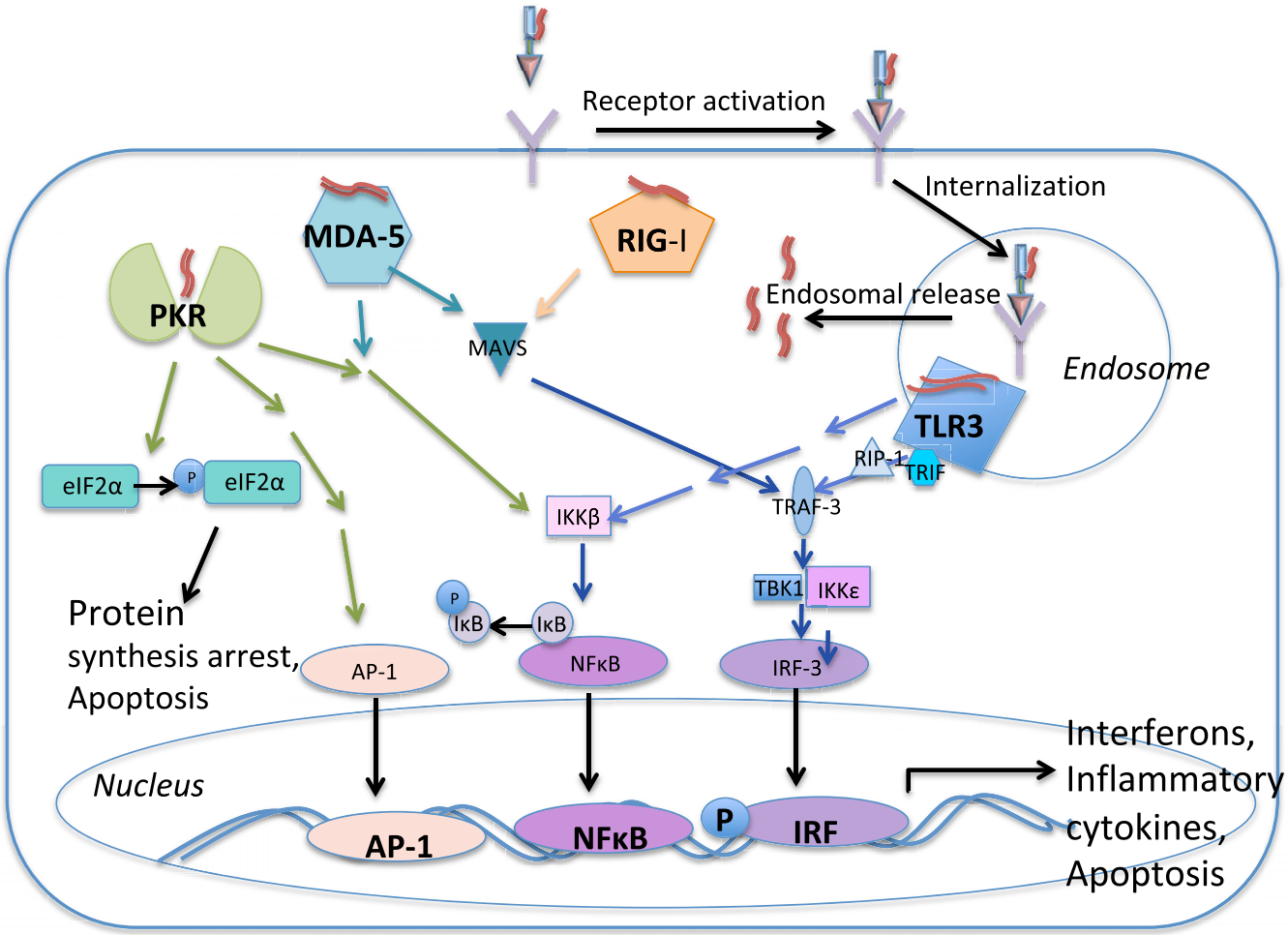

Fig. 3. Possible mechanisms of action of targeted polyIC. Targeted polyIC is internalized upon activation of the targeted receptor, which is overexpressed on the tumor cells. The double-stranded polyIC is expected to activate TLR3, which is mainly found in endosomes. TLR3, via its adaptor Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF), induces the phosphorylation of IFN Regulating Factor 3 (IRF3) and its translocation into the nucleus, as well as the phosphorylation of IKK and nuclear translocation of NFκB. IRF3 and NF-κB activate transcription of IFN-α, IFN-β, and proinflammatory cytokines such as TNF-α. Furthermore, upon endosomal release, polyIC can activate the cytoplasmic dsRNA receptors, PKR, MDA-5, and RIG-1. PKR and TLR3 also activate the MAPK pathway, resulting in activation of AP-1 and apoptosis. PKR directly phosphorylates eIF2-α, arresting protein synthesis. IFN production leads to further feedback activation of the "antiviral" response. Thus, polyIC induces the production of type I IFNs and inflammatory cytokines, leading to apoptosis and immune recruitment by multiple pathways.

The targeted polyIC strategy is different in concept from the tyrphostins we developed earlier: tyrphostins inhibit the kinase activity of the receptor, and we have learned that kinase inhibition is only effective when the cancer is strongly addicted to the kinase (as in the case of chronic CML). Our new strategy utilizes the receptor kinase as a Trojan horse, to deliver polyIC into the cancer cell. Upon binding, the vector-bound receptor is internalized, together with its polyIC cargo. Internalization of dsRNA induces the tumor to self-destruct by apoptosis, while activating immune modulatory pathways, leading to cytokine and chemokine induction. In addition to type I IFNs, we have detected RANTES, IP-10, GRO-α, IL-2, TNF-α, and IFN-γ (99, 106, 107). These molecules generate a "bystander effect" against the tumor: They are cytotoxic against tumor cells that do not themselves overexpress the receptor, boost tumor immunogenicity by increasing the expression of MHC-1 and tumor-specific antigens, and attract immune cells such as T cells and NK cells, which mount an attack against the tumor (100, 106, 108) (Fig. 4).

The targeted polyIC approach is valid for any cancer that is characterized by receptor overexpression in at least some of its cells. A vector homing to EGFR-overexpressing tumors was effective in clearing disseminated EGFR-overexpressing tumors from tumor-bearing mice (100). Only the cancerous cells are affected: Nontumor cells cannot internalize large amounts of polyIC, because they have far lower levels of EGFR, and they are far less sensitive to stress caused by the secreted cytokines (109).

By changing the "homing head" of our vectors, we have targeted HER-2–overexpressing breast cancer and prostate-specific membrane antigen (PSMA)–overexpressing prostate cancer xenografts (106–108). The involvement of multiple pathways in the response to targeted polyIC, the induction of a direct apoptotic effect that leads to rapid cell death, and the recruitment of the innate and adaptive immune systems to attack heterogeneous tumors and hunt out even distant metastases have combined to produce very impressive preclinical results (100, 106, 107). We believe that this strategy will be a worthwhile addition to the anticancer arsenal.

The Future of Cancer Therapy

Decades ago, an "abscopal effect," whereby radiation of a tumor at one site leads to regression of a tumor at another site, was recognized (110). Similarly, chemotherapy and targeted cytotoxic therapies can also induce antitumor immunity (111). Combining

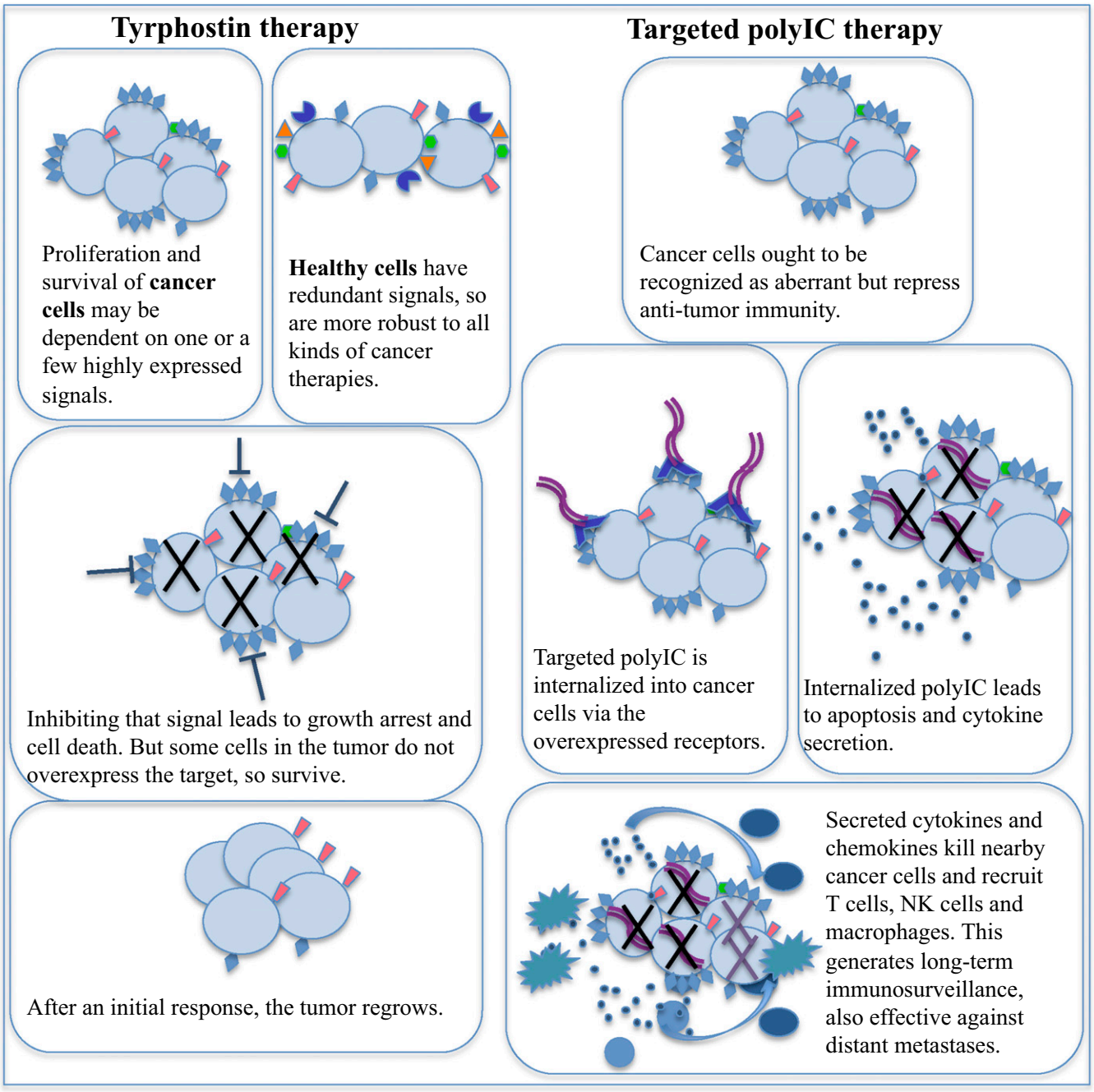

Fig. 4. The advantages of the targeted polyIC approach to tumor therapy.

ablation therapy of tumors with immunotherapy enhances the immunostimulating response and has synergistic effects for curative metastatic cancer treatment. Current efforts are aimed at finding the most effective combinations of chemotherapy, radiation, targeted therapy, and immunotherapy (112–117).

Our targeted polyIC strategy couples local cytotoxicity with activation of immune surveillance. We are also examining combinations of targeted polyIC with other forms of cancer treatment. If we have learned one thing over the years, it is that cancer growth and metastasis will only be controlled by a multifaceted approach.

ACKNOWLEDGMENTS. This work was supported by Israel Science Foundation Grant 1556/13.

1. Hunter T, Sefton BM (1980) Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc Natl Acad Sci USA* 77:1311–1315.
2. Levinson AD, Oppermann H, Varmus HE, Bishop JM (1980) The purified product of the transforming gene of avian sarcoma virus phosphorylates tyrosine. *J Biol Chem* 255:11973–11980.
3. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* 61:203–212.
4. Huggins C (1941) Studies on prostatic cancer. *Arch Surg* 43:209–223.
5. Jordan VC (2003) Tamoxifen: A most unlikely pioneering medicine. *Nat Rev Drug Discov* 2:205–213.
6. Atlas D, Levitzki A (1976) An irreversible blocker for the beta-adrenergic receptor. *Biochem Biophys Res Commun* 69:397–403.
7. Atlas D, Levitzki A (1977) Probing of beta-adrenergic receptors by novel fluorescent beta-adrenergic blockers. *Proc Natl Acad Sci USA* 74:5290–5294.

8. Gazit A, Yaish P, Gilon C, Levitzki A (1989) Tyrphostins I: Synthesis and biological activity of protein tyrosine kinase inhibitors. *J Med Chem* 32:2344–2352.
9. Gazit A, et al. (1991) Tyrphostins. 2. Heterocyclic and alpha-substituted benzylidenemalononitrile tyrphostins as potent inhibitors of EGF receptor and ErbB2/neu tyrosine kinases. *J Med Chem* 34:1896–1907.
10. Shechter Y, et al. (1989) Inhibition of insulin-dependent lipogenesis and anti-lipolysis by protein tyrosine kinase inhibitors. *EMBO J* 8:1671–1676.
11. Yaish P, Gazit A, Gilon C, Levitzki A (1988) Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science* 242:933–935.
12. Anafi M, Gazit A, Gilon C, Ben-Neriah Y, Levitzki A (1992) Selective interactions of transforming and normal abl proteins with ATP, tyrosine-copolymer substrates, and tyrphostins. *J Biol Chem* 267:4518–4523.
13. Anafi M, Gazit A, Zehavi A, Ben-Neriah Y, Levitzki A (1993) Tyrphostin-induced inhibition of p210bcr-abl tyrosine kinase activity induces K562 to differentiate. *Blood* 82:3524–3529.
14. Kovalenko M, et al. (1994) Selective platelet-derived growth factor receptor kinase blockers reverse sis-transformation. *Cancer Res* 54:6106–6114.
15. Gazit A, et al. (1996) Tyrphostins. 5. Potent inhibitors of platelet-derived growth factor receptor tyrosine kinase: Structure-activity relationships in quinoxalines, quinolines, and indole tyrphostins. *J Med Chem* 39:2170–2177.
16. Strawn LM, et al. (1996) Flk-1 as a target for tumor growth inhibition. *Cancer Res* 56: 3540–3545.
17. Meydan N, et al. (1996) Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. *Nature* 379:645–648.
18. Párrizas M, Gazit A, Levitzki A, Wertheimer E, LeRoith D (1997) Specific inhibition of insulin-like growth factor-1 and insulin receptor tyrosine kinase activity and biological function by tyrphostins. *Endocrinology* 138:1427–1433.
19. Blum G, Gazit A, Levitzki A (2003) Development of new insulin-like growth factor-1 receptor kinase inhibitors using catechol mimics. *J Biol Chem* 278:40442–40454.
20. Levitzki A, Mishani E (2006) Tyrphostins and other tyrosine kinase inhibitors. *Annu Rev Biochem* 75:93–109.
21. Druker BJ, et al. (1996) Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 2:561–566.
22. Kantarjian H, et al.; International STI571 CML Study Group (2002) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 346:645–652.
23. Druker BJ, et al. (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344:1038–1042.
24. Druker BJ (2002) STI571 (Gleevec) as a paradigm for cancer therapy. *Trends Mol Med* 8(Suppl 4):S14–S18.
25. Levitzki A, Gazit A (1995) Tyrosine kinase inhibition: An approach to drug development. *Science* 267:1782–1788.
26. Jänne PA, Engelman JA, Johnson BE (2005) Epidermal growth factor receptor mutations in non-small-cell lung cancer: Implications for treatment and tumor biology. *J Clin Oncol* 23:3227–3234.
27. Kennedy JA, Hobbs G, Kennedy JA (2018) Tyrosine kinase inhibitors in the treatment of chronic-phase CML: Strategies for frontline decision-making. *Curr Hematol Malig Rep* 13:202–211.
28. Mayekar MK, Bivona TG (2017) Current landscape of targeted therapy in lung cancer. *Clin Pharmacol Ther* 102:757–764.
29. Tang ZHZ-H, Lu J-JJJ (2018) Osimertinib resistance in non-small cell lung cancer: Mechanisms and therapeutic strategies. *Cancer Lett* 420:242–246.
30. Flaherty KT (2006) Chemotherapy and targeted therapy combinations in advanced melanoma. *Clin Cancer Res* 12:2366s–2370s.
31. Flaherty K (2008) Combination therapy: Key data and ongoing studies combining targeted and cytotoxic agents. *Community Oncol* 5:23–30.
32. Spano D, Heck C, De Antonellis P, Christofori G, Zollo M (2012) Molecular networks that regulate cancer metastasis. *Semin Cancer Biol* 22:234–249.
33. Puram SV, et al. (2017) Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck article single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell* 171:1611.e1–1611.e24.
34. Lawson DA, Kessenbrock K, Davis RT, Pervolarakis N, Werb Z (2018) Tumour heterogeneity and metastasis at single-cell resolution. *Nat Cell Biol* 20:1349–1360.
35. Petrelli A, Giordano S (2008) From single- to multi-target drugs in cancer therapy: When aspecificity becomes an advantage. *Curr Med Chem* 15:422–432.
36. Lee SJ, Wang JYJ (2009) Exploiting the promiscuity of imatinib. *J Biol* 8:30.
37. Reuveni H, et al. (2013) Therapeutic destruction of insulin receptor substrates for cancer treatment. *Cancer Res* 73:4383–4394.
38. Flashner-Abramson E, et al. (2016) Targeting melanoma with NT157 by blocking Stat3 and IGF1R signaling. *Oncogene* 35:2675–2680.
39. Su S-P, et al. (2018) Impact of the anticancer drug NT157 on tyrosine kinase signaling networks. *Mol Cancer Ther* 17:931–942.
40. Yu H, Kortylewski M, Pardoll D (2007) Crosstalk between cancer and immune cells: Role of STAT3 in the tumour microenvironment. *Nat Rev Immunol* 7:41–51.
41. Bournazou E, Bromberg J (2013) Targeting the tumor microenvironment: JAK-STAT3 signaling. *JAK-STAT* 2:e23828.
42. Wong ALA, et al. (2017) Do STAT3 inhibitors have potential in the future for cancer therapy? *Expert Opin Investig Drugs* 26:883–887.
43. Sanchez-Lopez E, et al. (2016) Targeting colorectal cancer via its microenvironment by inhibiting IGF-1 receptor-insulin receptor substrate and STAT3 signaling. *Oncogene* 35:2634–2644.
44. Dunn GP, Old LJ, Schreiber RD (2004) The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 21:137–148.
45. Schreiber RD, Old LJ, Smyth MJ (2011) Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science* 331:1565–1570.
46. Wiemann B, Starnes CO (1994) Coley's toxins, tumor necrosis factor and cancer research: A historical perspective. *Pharmacol Ther* 64:529–564.
47. Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 6:443–446.
48. Nasiri H, Valedkarimi Z, Aghebati-Maleki L, Majidi J (2018) Antibody-drug conjugates: Promising and efficient tools for targeted cancer therapy. *J Cell Physiol* 233: 6441–6457.
49. Polakis P (2016) Antibody drug conjugates for cancer therapy. *Pharmacol Rev* 68:3–19.
50. Nierkens S, Janssen EM (2011) Harnessing dendritic cells for tumor antigen presentation. *Cancers (Basel)* 3:2195–2213.
51. Sánchez-Paulete AR, et al. (2017) Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy. *Ann Oncol* 28:xii44–xii55.
52. Gardner A, Ruffell B (2016) Dendritic cells and cancer immunity. *Trends Immunol* 37: 855–865.
53. Eshhar Z (2008) The T-body approach: Redirecting T cells with antibody specificity. *Handb Exp Pharmacol* 181:329–342.
54. Kochenderfer JN, et al. (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 119:2709–2720.
55. Porter DL, Levine BL, Kalos M, Bagg A, June CH (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 365:725–733.
56. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC (2018) CAR T cell immunotherapy for human cancer. *Science* 359:1361–1365.
57. Yu S, et al. (2017) Chimeric antigen receptor T cells: A novel therapy for solid tumors. *J Hematol Oncol* 10:78.
58. Gomes-Silva D, Ramos CA (February 2, 2018) Cancer immunotherapy using CAR-T cells: From the research bench to the assembly line. *Biotechnol J*10.1002/biot.201700097.
59. Kato S, et al. (2017) Hyperprogressors after immunotherapy: Analysis of genomic alterations associated with accelerated growth rate. *Clin Cancer Res* 23:4242–4250.
60. Champiat S, et al. (2017) Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clin Cancer Res* 23:1920–1928.
61. Wartewig T, et al. (2017) PD-1 is a haploinsufficient suppressor of T cell lymphomagenesis. *Nature* 552:121–125.
62. Rieth J, Subramanian S (2018) Mechanisms of intrinsic tumor resistance to immunotherapy. *Int J Mol Sci* 19:1393.
63. Jenkins RW, Barbie DA, Flaherty KT (2018) Mechanisms of resistance to immune checkpoint inhibitors. *Br J Cancer* 118:9–16.
64. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A (2017) Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 168:707–723.
65. Schumacher TN, Schreiber RD (2015) Neoantigens in cancer immunotherapy. *Science* 348:69–74.
66. Llosa NJ, et al. (2015) The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 5:43–51.
67. Lee JJ, Chu E (2018) Recent advances in the clinical development of immune checkpoint blockade therapy for mismatch repair proficient (pMMR)/non-MSI-H metastatic colorectal cancer. *Clin Colorectal Cancer* 17:258–273.
68. Horita H, Law A, Hong S, Middleton K (2017) Identifying regulatory posttranslational modifications of PD-L1: A focus on monoubiquitinaton. *Neoplasia* 19:346–353.
69. Restifo NP, et al. (1996) Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J Natl Cancer Inst* 88:100–108.
70. Sucker A, et al. (2014) Genetic evolution of T-cell resistance in the course of melanoma progression. *Clin Cancer Res* 20:6593–6604.
71. Sade-Feldman M, et al. (2017) Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun* 8:1136.
72. Peng W, et al. (2016) Loss of PTEN promotes resistance to T cell–mediated immunotherapy. *Cancer Discov* 6:202–216.
73. George S, et al. (2017) Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma. *Immunity* 46: 197–204.
74. Anderson AC (2014) Tim-3: An emerging target in the cancer immunotherapy landscape. *Cancer Immunol Res* 2:393–398.
75. Sakuishi K, et al. (2010) Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 207:2187–2194.
76. Fourcade J, et al. (2010) Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific $CD8^+$ T cell dysfunction in melanoma patients. *J Exp Med* 207:2175–2186.
77. Ngiow SF, et al. (2011) Anti-TIM3 antibody promotes T cell IFN-γ-mediated antitumor immunity and suppresses established tumors. *Cancer Res* 71:3540–3551.
78. Koyama S, et al. (2016) Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun* 7:10501.
79. Arlauckas SP, et al. (2017) In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. *Sci Transl Med* 9:eaal3604.
80. Thallinger C, et al. (2018) Review of cancer treatment with immune checkpoint inhibitors: Current concepts, expectations, limitations and pitfalls. *Wien Klin Wochenschr* 130:85–91.
81. Jerby-Arnon L, et al. (2018) A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. *Cell* 175:984–997.e24.
82. Sade-Feldman M, et al. (2018) Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 175:998–1013.e20.

83. Hugo W, et al. (2016) Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 165:35–44.
84. Desjardins A, et al. (2018) Recurrent glioblastoma treated with recombinant poliovirus. *N Engl J Med* 379:150–161.
85. Rizza P, Moretti F, Capone I, Belardelli F (2015) Role of type I interferon in inducing a protective immune response: Perspectives for clinical applications. *Cytokine Growth Factor Rev* 26:195–201.
86. Di Trolio R, Simeone E, Di Lorenzo G, Buonerba C, Ascierto PA (2015) The use of interferon in melanoma patients: A systematic review. *Cytokine Growth Factor Rev* 26:203–212.
87. Ortiz A, Fuchs SY (2017) Anti-metastatic functions of type 1 interferons: Foundation for the adjuvant therapy of cancer. *Cytokine* 89:4–11.
88. Goldstein D, Laszlo J (1986) Interferon therapy in cancer: From imaginon to interferon. *Cancer Res* 46:4315–4329.
89. Rosenberg SA (2014) IL-2: The first effective immunotherapy for human cancer. *J Immunol* 192:5451–5458.
90. Jiang T, Zhou C, Ren S (2016) Role of IL-2 in cancer immunotherapy. *OncoImmunology* 5:e1163462.
91. Holmgaard RB, Zamarin D, Lesokhin A, Merghoub T, Wolchok JD (2016) Targeting myeloid-derived suppressor cells with colony stimulating factor-1 receptor blockade can reverse immune resistance to immunotherapy in indoleamine 2,3-dioxygenase-expressing tumors. *EBioMedicine* 6:50–58.
92. Neubert NJ, et al. (2018) T cell-induced CSF1 promotes melanoma resistance to PD1 blockade. *Sci Transl Med* 10:eaan3311.
93. Mao Y, et al. (2016) Targeting suppressive myeloid cells potentiates checkpoint inhibitors to control spontaneous neuroblastoma. *Clin Cancer Res* 22:3849–3859.
94. Sato-Kaneko F, et al. (2017) Combination immunotherapy with TLR agonists and checkpoint inhibitors suppresses head and neck cancer. *JCI Insight* 2:1–18.
95. Schmidt M, Kapp K, Volz B, Oswald D, Wittig B (2017) TLR9 agonist lefitolimod to improve antitumor effect of checkpoint inhibitors in vivo. *J Clin Oncol* 35(Suppl 15): e14625.
96. Burdelya L, et al. (2002) Combination therapy with AG-490 and interleukin 12 achieves greater antitumor effects than either agent alone. *Mol Cancer Ther* 1:893–899.
97. Lasek W, Zagożdżon R, Jakobisiak M (2014) Interleukin 12: Still a promising candidate for tumor immunotherapy? *Cancer Immunol Immunother* 63:419–435.
98. Wang P, et al. (2017) Re-designing Interleukin-12 to enhance its safety and potential as an anti-tumor immunotherapeutic agent. *Nat Commun* 8:1395.
99. Edinger N, et al. (2016) Targeting polyIC to EGFR over-expressing cells using a dsRNA binding protein domain tethered to EGF. *PLoS One* 11:e0162321.
100. Shir A, Ogris M, Roedl W, Wagner E, Levitzki A (2011) EGFR-homing dsRNA activates cancer-targeted immune response and eliminates disseminated EGFR-overexpressing tumors in mice. *Clin Cancer Res* 17:1033–1043.
101. Gantier MP, Williams BRG (2007) The response of mammalian cells to double-stranded RNA. *Cytokine Growth Factor Rev* 18:363–371.
102. Balachandran S, et al. (2000) Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. *Immunity* 13:129–141.
103. Palchetti S, et al. (2015) Transfected poly(I:C) activates different dsRNA receptors, leading to apoptosis or immunoadjuvant response in androgen-independent prostate cancer cells. *J Biol Chem* 290:5470–5483.
104. Chawla-Sarkar M, et al. (2003) Apoptosis and interferons: Role of interferon-stimulated genes as mediators of apoptosis. *Apoptosis* 8:237–249.
105. Shekarian T, et al. (2017) Pattern recognition receptors: Immune targets to enhance cancer immunotherapy. *Ann Oncol* 28:1756–1766.
106. Zigler M, et al. (2016) HER2-targeted polyinosine/polycytosine therapy inhibits tumor growth and modulates the tumor immune microenvironment. *Cancer Immunol Res* 4:688–697.
107. Langut Y, et al. (2017) PSMA-targeted polyinosine/polycytosine vector induces prostate tumor regression and invokes an antitumor immune response in mice. *Proc Natl Acad Sci USA* 114:13655–13660.
108. Langut Y, et al. (2017) PSMA-homing dsRNA chimeric protein vector kills prostate cancer cells and activates anti-tumor bystander responses. *Oncotarget* 8:24046–24062.
109. Benhar M, Engelberg D, Levitzki A (2002) ROS, stress-activated kinases and stress signaling in cancer. *EMBO Rep* 3:420–425.
110. Mole RH (1953) Whole body irradiation; radiobiology or medicine? *Br J Radiol* 26: 234–241.
111. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G (2015) Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* 28:690–714.
112. Ngwa W, et al. (2018) Using immunotherapy to boost the abscopal effect. *Nat Rev Cancer* 18:313–322.
113. Gotwals P, et al. (2017) Prospects for combining targeted and conventional cancer therapy with immunotherapy. *Nat Rev Cancer* 17:286–301.
114. Palucka AK, Coussens LM (2016) The basis of oncoimmunology. *Cell* 164:1233–1247.
115. Patel SA, Minn AJ (2018) Combination cancer therapy with immune checkpoint blockade: Mechanisms and strategies. *Immunity* 48:417–433.
116. Ribas A, Wolchok JD (2013) Combining cancer immunotherapy and targeted therapy. *Curr Opin Immunol* 25:291–296.
117. Wargo JA, Reuben A, Cooper ZA, Oh KS, Sullivan RJ (2015) Immune effects of chemotherapy, radiation, and targeted therapy and opportunities for combination with immunotherapy. *Semin Oncol* 42:601–616.