

Type I interferons in anticancer immunity

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Abstract | Type I interferons (IFNs) are known for their key role in antiviral immune responses. In this Review, we discuss accumulating evidence indicating that type I IFNs produced by malignant cells or tumour-infiltrating dendritic cells also control the autocrine or paracrine circuits that underlie cancer immunosurveillance. Many conventional chemotherapeutics, targeted anticancer agents, immunological adjuvants and oncolytic viruses are only fully efficient in the presence of intact type I IFN signalling. Moreover, the intratumoural expression levels of type I IFNs or of IFN-stimulated genes correlate with favourable disease outcome in several cohorts of patients with cancer. Finally, new anticancer immunotherapies are being developed that are based on recombinant type I IFNs, type I IFN-encoding vectors and type I IFN-expressing cells.

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Type I interferons (IFNs) were first discovered more than half a century ago as the factors underlying viral interference — that is, the ability of a primary viral infection to render cells resistant to a second distinct virus¹. Type I IFNs comprise IFN α proteins (a class of homologous proteins that are encoded by 13 distinct genes in humans, *IFNA1* to *IFNA13*), IFN β (that is encoded by a single gene in humans and mice, *IFNB1*) and other, less investigated IFNs, such as IFN ϵ , IFN κ and IFN ω , which will not be discussed in this Review^{2,3}. Type I IFNs are produced by multiple cell types following activation of pattern recognition receptors (PRRs) (BOX 1). PRRs respond to viral or bacterial components and also to endogenous molecules found in ectopic locations (such as cytosolic DNA and extracellular DNA and RNA)⁴. Type I IFNs signal via a homodimeric IFN α/β receptor 1 (IFNAR1), which has a particularly high affinity for IFN β , or via an IFNAR1–IFNAR2 heterodimer, which binds all type I IFNs. The activation of these receptors elicits many immunostimulatory effects (BOX 2) following the transcriptional upregulation of IFN-stimulated genes (ISGs), some of which are also responsible for viral interference^{5–7}.

In this Review, we discuss the growing body of evidence suggesting that type I IFNs have a major role not only in antiviral immune responses but also in the natural and the therapy-induced immunological control of virus-unrelated malignancies. These advances have far-reaching implications for tumour immunology, drug development and clinical oncology.

Type I IFNs in cancer immunosurveillance

Type I IFNs are known to mediate antineoplastic effects against several malignancies, which is a clinically relevant activity that has been attributed to their immunostimulatory functions⁸. However, the precise role of type I IFNs in the natural immune response to cancer has only begun to be understood in the past decade. Experimental data strongly suggest the existence of a process whereby the immune system, in the absence of external manipulations, protects the host against oncogenesis and controls the immunological features of developing tumours⁹. This process, which has been called cancer immunoeediting, consists of three phases: first, the elimination of malignant cells by the immune system; second, the establishment of an equilibrium between genetically unstable malignant cells and the immune system, which reflects the immunoeediting imposed by the immune system on cancer cells; and third, the escape of neoplastic cell variants with reduced immunogenicity, which ultimately form clinically manifest neoplasms¹⁰. Type I IFNs intervene in all of these phases^{11,12}.

At least some cell types produce type I IFNs and/or respond to them to avoid neoplastic transformation. Indeed, the absence of *Ifnb1* or *Ifnar1* predisposes mouse embryonic fibroblasts to cellular transformation¹³, and some viral oncoproteins interfere with the functions of ISGs¹⁴. The tissue-specific deletion of *Ifnar1* from intestinal epithelial cells increases tumour burden in mice treated with the colitis-inducing agent dextran sodium

Box 1 | Sources and signals underlying type I IFN production

Plasmacytoid DCs (pDCs) produce high amounts of type I interferons (IFNs) following stimulation of Toll-like receptor 7 (TLR7) and TLR9, which detect viral RNA and DNA molecules, respectively, that have been endocytosed or sequestered by autophagy¹⁰³. pDCs are also capable of sensing host-derived nucleic acids; for instance, this occurs in the context of skin wounds¹⁰⁴. In this setting, the host DNA binds to cathelicidin peptides, which promote the access of the nucleic acids to intracellular TLRs and hence contribute to early inflammatory responses and re-epithelialization¹⁰⁴.

Other sources of type I IFNs are well characterized. For instance, CD141⁺ conventional DCs are prominent producers of IFN α in humanized mice following administration of polyinosinic–polycytidylic acid (poly:I:C)¹⁰⁵. Moreover, almost any cell in the body can synthesize type I IFNs upon activation of cytosolic receptors for double-stranded RNA (dsRNA), particularly the RNA helicases retinoic acid-inducible gene protein I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5). RIG-I and MDA5 signal through mitochondrial antiviral-signalling protein (MAVS) and TANK-binding kinase 1 (TBK1) to activate the IFN-regulatory factor 3 (IRF3)-dependent transcription of type I IFN-coding genes². Similarly, type I IFN can be produced upon the activation of stimulator of IFN genes protein (STING) and MAVS by the bacterial second messenger cyclic di-GMP²⁴. STING is also required for apoptotic thymocytes to synthesize immunosuppressive factors, such as indoleamine 2,3-dioxygenase 1 (IDO1), interleukin-10 (IL-10) and transforming growth factor- β 1 (TGF β 1), *in vivo*¹⁰⁰, probably owing to its ability to drive type I IFN production. Consistent with this idea, type I IFNs stimulate the release of IL-10 from regulatory T (T_{Reg}) cells and T regulatory type 1 cells (T_R1 cells) in mice and humans^{106,107}. Of note, the production of type I IFNs can be amplified by a positive feedback loop that involves the transactivation of *IRF7* and, at least in DCs, *IRF8* in response to IFN α/β receptor (IFNAR) signalling.

Cell death can influence immune responses as it is associated with the emission of danger signals that activate antigen-presenting cells (APCs). Several possible pattern recognition receptors (PRRs) — including STING and TLRs, as well as C-type lectin domain family 9 member A (CLEC9A) — and the autophagy-facilitated transfer of dead cell-associated antigens to APCs may be involved in the induction of type I IFNs *in vivo*^{25,108,109}. For instance, tumour cell-derived DNA seems to trigger the production of type I IFNs in CD11c⁺ tumour-infiltrating DCs through cyclic GMP–AMP synthase (cGAS), STING and IRF3, thereby priming CTLs specific for tumour-associated antigens⁴⁷. Moreover, cGAS and STING have been suggested to underlie the production of type I IFNs by cancer cells in response to mitochondrial outer membrane permeabilization (especially when apoptotic caspases are inhibited), a process that coincides with the release of mitochondrial DNA into the cytosol^{110–112}.

Pattern recognition receptors

(PRRs). Evolutionarily old receptors expressed by cells of the innate immune system. PRRs detect viral and bacterial components that are commonly referred to as microorganism-associated molecular patterns (MAMPs), as well as endogenous molecules known as damage-associated molecular patterns (DAMPs). PRRs constitute key sensors of danger.

T regulatory type 1 cells

(T_R1 cells). A subset of immunosuppressive CD4⁺ T cells that downregulate T helper 1 (T_H1) and T_H2 cell responses *in vitro* and *in vivo* by a contact-independent mechanism that is mediated by the secretion of soluble interleukin-10 and transforming growth factor- β 1.

sulfate (DSS) plus the carcinogen azoxymethane (AOM)¹⁵. Furthermore, signal transducer and activator of transcription 1 (STAT1; which operates downstream of IFNARs) is frequently not expressed in human oestrogen receptor 1 (ESR1)-expressing breast carcinomas, and mice lacking *Stat1* spontaneously develop ESR1⁺ mammary tumours¹⁶. Taken together, these observations suggest that both viral and non-viral instances of oncogenesis are inhibited by type I IFN signalling in premalignant cells.

The metastatic dissemination of human breast carcinomas to the bone is generally coupled with a deficient production of type I IFNs by cancer cells, and this has been attributed to decreased expression levels of IFN-regulatory factor 7 (IRF7)¹⁷. Consistent with this idea, enforced re-expression of IRF7 within IRF7-deficient neoplastic cells (which restores type I IFN secretion) or the administration of recombinant IFN α inhibited bone metastases in a mouse model of mammary oncogenesis. Conversely, metastatic dissemination was accelerated in *Ifnar1*^{-/-} mice, as well as in mice depleted of natural killer (NK) cells and T cells¹⁷. Thus, in this model, tumour-derived type I IFNs inhibited metastatic dissemination through IFNAR1 expressed by host immune cells.

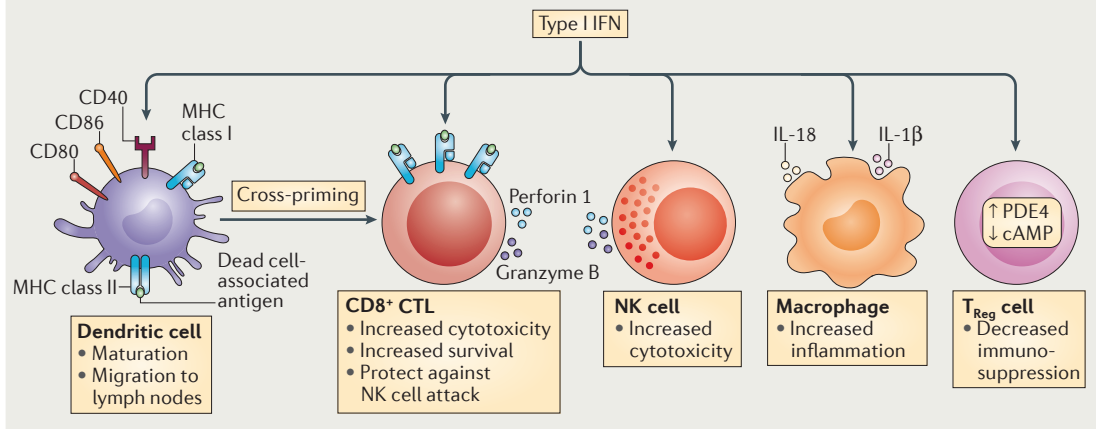
The knockout of *Ifnar1* or *Ifnar2* increases the incidence of methylcholanthrene (MCA)-induced fibrosarcomas in mice^{11,18}. In this context, IFNAR1 must be expressed by the radiosensitive haematopoietic cell compartment to participate in immunosurveillance. Moreover, some MCA-induced *Ifnar1*^{-/-} fibrosarcoma cells were unable to form tumours following transfer to wild-type mice because they were rejected by the host (in which type I IFN signalling is intact)¹¹. Taken together with the results obtained from models of mammary carcinogenesis¹⁷, these findings suggest that in many instances cancer immunosurveillance does not rely on the induction of IFNAR1 signalling in cancer cells. Accordingly, *Ifnar1*^{-/-} CD8 α ⁺ dendritic cells (CD8 α ⁺ DCs) are deficient in antigen cross-presentation, and mice lacking *Ifnar1* only in this cellular compartment fail to reject highly immunogenic malignant cells¹⁹. These results indicate that type I IFN signalling is a crucial component of the innate immune response to transformed cells. Of note, this study¹⁹ identified a link between type I IFNs and CD8 α ⁺ DCs that could explain the requirement for this antigen-presenting cell (APC) subset in the spontaneous cross-priming of tumour-specific CD8⁺ cytotoxic T lymphocytes (CTLs) *in vivo*²⁰. Interestingly, Reis e Sousa and colleagues^{21,22} showed a role for C-type lectin domain family 9 member A (CLEC9A) — which is a plasma membrane receptor highly expressed by CD8 α ⁺ DCs — in the cross-presentation of antigens from dying and virus-infected cells, making it logical to pursue a connection between this system and type I IFN signalling. However, it is not yet known whether CLEC9A is required for cancer immunosurveillance and whether it functions upstream or downstream of IFNAR in CD8 α ⁺ DCs. Another C-type lectin receptor expressed by APCs, CLEC7A (also known as dectin 1), might be implicated in innate anticancer immune responses. Chiba *et al.*²³ described a “tumour-associated molecular pattern” consisting of cancer cell-specific changes in surface *N*-glycans that engage CLEC7A on DCs and macrophages. The activation of CLEC7A has been shown to induce an IRF5-dependent signalling pathway that culminates in NK cell-dependent tumour control²³.

Stimulator of IFN genes protein (STING; encoded by *TMEM173*) is a major regulator of innate immune responses to pathogens and is the main PRR that induces type I IFN production by DCs²⁴. DCs from *Tmem173*^{-/-} mice are defective at priming CTLs specific for tumour-associated antigens (TAAs), whereas DCs from mice that lack other PRRs or PRR-related signal transducers — such as myeloid differentiation primary response protein 88 (MYD88), TIR domain-containing adaptor protein inducing IFN β (TRIF; also known as TICAM1) and mitochondrial antiviral-signalling protein (MAVS), — retain their CTL-priming ability²⁵. STING has an important role in antigen presentation by plasmacytoid DCs (pDCs), which have been shown to secrete high levels of type I IFNs in the T cell zones of lymphatic tissue associated with a range of different tumours^{26,27}. Thus, pDCs may constitute the major source of type I IFNs that support the priming of TAA-specific immune responses, but this hypothesis has not yet been

Box 2 | Signals elicited by type I IFNs

Upon ligation, the IFN α/β receptor 1 (IFNAR1)–IFNAR2 heterodimer activates tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1), which results in the recruitment of signal transducer and activator of transcription 1 (STAT1) and STAT2 to the cytoplasmic tail of the receptor and in the formation of STAT1–STAT2 heterodimers that can migrate to the nucleus. Therein, STAT1–STAT2 heterodimers associate with interferon (IFN)–regulatory factor 9 (IRF9) to form the heterotrimeric transcriptional complex known as IFN-stimulated gene factor 3 (ISGF3). Upon binding to specific DNA response elements, ISGF3 transactivates IFN-inducible genes². Type I IFNs can also cause the stabilization of other transcriptionally active STAT homodimers and heterodimers, the CRK-like protein (CRKL)–STAT5 heterodimer and nuclear factor- κ B (NF- κ B). Moreover, type I IFN signalling can trigger the phosphoinositide 3-kinase (PI3K) signal transduction cascade¹¹³. Finally, type I IFNs can promote the activation of the guanine nucleotide-exchange factor VAV1, thereby initiating a broad response that involves multiple transcription factors, including (but not limited to) the STAT1–STAT2 heterodimer, ELK1, MYC and tumour protein p53 (TP53)⁵. IFN β (but not IFN α) may also bind to IFNAR1 homodimers, hence inducing a distinct set of signals independently of IFNAR2, JAK1 and STAT1 (REF. 6). This may be an explanation for biological differences between IFN α and IFN β that have not yet been extensively studied *in vivo*.

Type I IFNs support cytotoxic T lymphocytes (CTLs) by various mechanisms: first, they promote cross-priming by stimulating the maturation of dendritic cells (DCs), by enhancing their capacity to process and present dead cell-associated antigens, and by promoting their migration towards lymph nodes¹¹⁴; second, they boost immune effector functions by increasing the expression of perforin 1 and granzyme B¹¹⁵; and third, they promote the survival of memory CTLs⁶⁶. Moreover, type I IFNs can prevent the elimination of antigen-activated CD8⁺ CTLs by natural killer (NK) cells, as they reduce the ratio of activatory versus inhibitory NK cell receptor ligands expressed by CTLs^{116,117} and they stimulate the release of pro-inflammatory cytokines (such as interleukin-1 β (IL-1 β) and IL-18) by macrophages¹¹⁸. Finally, type I IFNs can inactivate the suppressive function of regulatory T (T_{Reg}) cells through a pathway that involves the activation of phosphodiesterase 4 (PDE4) and the consequent depletion of cyclic AMP (cAMP)¹¹⁹ (see the figure).



CD8 α^+ dendritic cells (CD8 α^+ DCs). A DC subset phenotypically characterized by the expression of *Cd8a* (in mice) and particularly efficient at cross-presentation: that is, at presenting extracellular antigens on MHC class I molecules to CD8⁺ cytotoxic T cells, rather than on MHC class II molecules to CD4⁺ T helper cells.

Cross-priming
The initiation of a CD8⁺ T cell response against an antigen that is not expressed by antigen-presenting cells (APCs). Cross-priming relies on the ability of some APCs to redirect internalized antigens to the MHC class I presentation pathway (cross-presentation).

Stimulator of IFN genes protein (STING). A protein of the endoplasmic reticulum membrane (encoded by *TMEM173*) that promotes the production of type I interferons (IFNs) in response to cyclic di-GMP and works as an adaptor in the signal transduction cascades induced by other cytosolic sensors of nucleic acids.

Plasmacytoid DCs (pDCs). A dendritic cell (DC) subset that is phenotypically characterized by reduced expression levels of CD11c and CD14 and that is particularly efficient at type I interferon production in response to several stimuli.

formally addressed. Irrespective of the source of type I IFNs, robust tumour infiltration by CTLs and NK cells correlates with spontaneous type I IFN production and a favourable prognosis in patients with melanoma^{26,28–30}.

The pDCs that infiltrate human breast carcinomas were shown to be defective at producing type I IFNs in response to Toll-like receptor 9 (TLR9) agonists compared with circulating pDCs³¹. Such a defect in type I IFN production may reflect the physical colocalization within the tumour bed and the numerical correlation of pDCs and immunosuppressive CD4⁺CD25⁺FOXP3⁺ regulatory T (T_{Reg}) cells. The selective suppression of type I IFN production in tumour-infiltrating pDCs endows them with the ability to support the proliferation of T_{Reg} cells *in vitro*, and this can be blocked by the administration of type I IFN³¹. These data support the existence of a mutually reinforcing immunosuppressive circuit that involves tumour-infiltrating pDCs and T_{Reg} cells; however, the molecular mechanisms that underlie such a circuit have yet to be elucidated. Irrespective of this, it seems that a type I IFN-related genetic signature identified by RNA sequencing predicts metastasis-free

survival in patients with breast cancer³². This corroborates the idea that intratumoural type I IFN signalling stimulates anticancer immunosurveillance and hence may improve disease outcome in patients with cancer.

Of note, innate immune cells may not be the only source of type I IFNs in the tumour microenvironment. Indeed, copy number loss of the *IFN* gene cluster on chromosome 9p21.3 — which includes *IFNB1* and the IFN-unrelated tumour suppressor genes cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and *CDKN2B* — in melanoma cells is associated with poor disease outcome³³. This indicates that malignant cells themselves can produce type I IFNs, which may support cancer immunosurveillance.

Thus, type I IFNs are involved in the innate immune response to developing malignancies by actively participating in cancer immunosurveillance. It remains to be determined which tumour-derived products and which signal transduction pathways underlie such an effect. Presumably, this process involves the death of a subset of tumour cells as the neoplastic lesion grows and evolves *in vivo*, which results in the production of type I IFNs.

Type I IFNs and anticancer therapies

Accumulating evidence indicates that the success of conventional chemotherapeutics, targeted anticancer agents, radiotherapy and immunotherapy relies on type I IFN signalling (FIG. 1).

In mouse models, IFNAR1-neutralizing monoclonal antibodies abolish the therapeutic effect of monoclonal antibodies that are specific for human epidermal growth factor receptor 2 (HER2; also known as ERBB2) or those specific for epidermal growth factor receptor (EGFR)^{34,35}. The efficacy of anthracycline-based chemotherapy against established transplanted tumours in mice is also lost upon co-administration of an IFNAR1-neutralizing monoclonal antibody³⁶. Genetic analyses revealed that IFNAR1 expressed by the cancer cells themselves (rather than by the host) supports the activity of anthracyclines in this experimental setting³⁶. It has been suggested that some immunogenic chemotherapeutics, including anthracyclines³⁷, promote the activation of TLR3 in mouse and human malignant cells by cancer cell-derived RNA, which results in the secretion of type I IFNs. Type I IFNs then activate an autocrine or

a paracrine IFNAR-dependent circuit that results in the expression of various ISGs, including CXC-chemokine ligand 10 (CXCL10; which is a potent chemoattractant for innate immune cells) and the antiviral factor MX dynamin-like GTPase 1 (MX1)³⁶. Importantly, increased expression levels of MX1 in biopsy samples from patients with breast carcinoma treated with anthracycline-based chemotherapy has been suggested to predict the likelihood of these individuals to respond to treatment³⁶.

The administration of cyclophosphamide (another chemotherapeutic with immunological off-target effects) to patients with haematological cancers causes transient changes in the gene expression profile of circulating leukocytes that peak approximately 2 days after chemotherapy³⁸. This cyclophosphamide-associated gene expression profile contains a type I IFN-related signature, as well as markers of a sterile inflammatory response^{39,40}. Similarly, cyclophosphamide causes the IFNAR1-dependent proliferation of CD8 α^+ CD11c $^+$ DCs in tumour-bearing mice⁴¹. These findings indicate that various chemotherapeutics that are commonly used in the clinic stimulate type I IFN signalling as part of their antineoplastic effects.

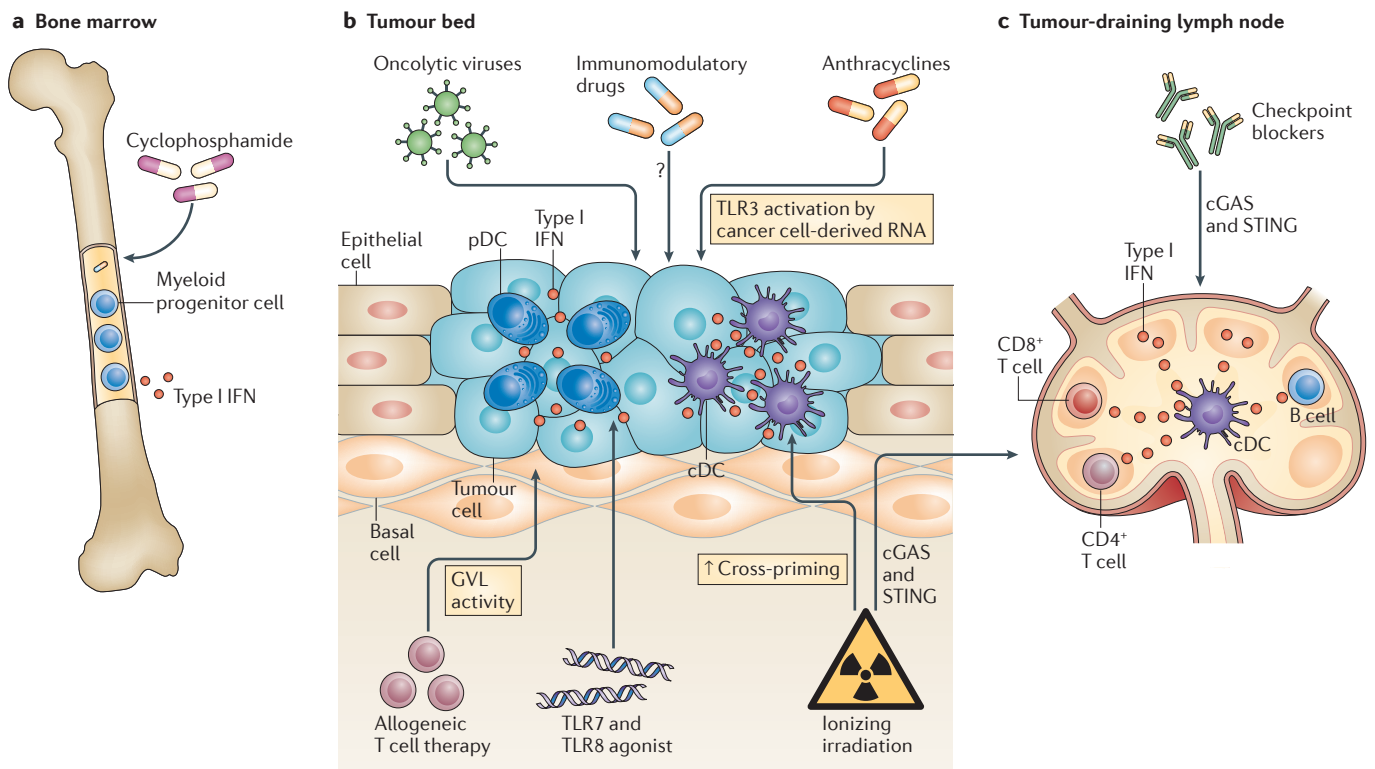


Figure 1 | Contribution of type I IFNs to the efficacy of anticancer therapy. The clinical activity of a wide range of chemotherapeutic, radiotherapeutic and immunotherapeutic interventions relies on the induction of type I interferon (IFN) signalling in malignant cells, tumour-infiltrating myeloid cells or within lymphoid organs. **a** | Cyclophosphamide stimulates the production of type I IFNs by myeloid progenitor cells in the bone marrow. **b** | Oncolytic viruses, Toll-like receptor 7 (TLR7) and TLR8 agonists, and anthracyclines (which promote the activation of TLR3 by cancer cell-derived RNA) induce the secretion of type I IFNs by cancer cells and/or by myeloid cells such as plasmacytoid dendritic cells (pDCs) and conventional DCs (cDCs) in the tumour bed. Type I IFN production is required for the clinical efficacy of these interventions.

Immunomodulatory drugs also stimulate the secretion of type I IFNs within neoplastic lesions, but whether this is essential for therapeutic responses remains to be determined. In addition, intratumoural type I IFN signalling contributes to the clinical efficacy of allogeneic T cell therapy, which mediates a graft-versus-leukaemia (GVL) effect, and of ionizing irradiation. The pattern recognition receptors underlying the ability of these anticancer interventions to stimulate type I IFN signalling have not been completely identified. **c** | Indirect evidence suggests that the therapeutic activity of checkpoint blockers and ionizing irradiation involves the production of type I IFNs within tumour-draining lymph nodes. This is dependent on cyclic GMP-AMP synthase (cGAS) and stimulator of IFN genes protein (STING).

Graft-versus-leukaemia (GVL). The process by which allogeneic haematopoietic stem cell grafts recognize (and eliminate) residual leukaemic cells in the host as a result of some degree of mismatch between minor histocompatibility antigens.

Cytotoxic T lymphocyte-associated protein 4 (CTLA4). A plasma membrane receptor of the immunoglobulin superfamily that is expressed by activated T cells. It is involved in the physiological extinction of immune responses but is also harnessed by malignant cells to establish an immunosuppressive tumour microenvironment.

Programmed cell death protein 1 (PD1). Plasma membrane receptor of the immunoglobulin superfamily expressed by activated T cells, B cells and macrophages. Similar to cytotoxic T lymphocyte-associated protein 4 (CTLA4), PD1 is harnessed by cancer cells for the establishment of local and systemic immunosuppression.

Immunomodulatory drugs (IMiDs). A group of molecules with immunomodulatory effects currently approved for the treatment of erythema nodosum leprosum (a complication of leprosy), multiple myeloma and myelodysplastic syndrome. IMiDs include thalidomide, lenalidomide and pomalidomide.

Anticancer virotherapy
A peculiar paradigm of anticancer immunotherapy based on the administration of natural or genetically modified viruses that selectively kill malignant cells.

Pegylated IFN
Recombinant interferon (IFN) modified by the addition of a polyethylene glycol (PEG) moiety. This modification improves the half-life of recombinant IFN in the circulation.

Radiation therapy induces the production of IFN β by the myeloid (rather than the malignant) compartment of B16F1 melanomas, and the antineoplastic effects of irradiation are lost in *Ifnar1*^{-/-} mice, as well as in mice bearing *Ifnar1*^{-/-} haematopoietic cells⁴². This phenomenon correlates with the ability of radiation therapy to boost cross-priming by tumour-infiltrating IFNAR1-expressing CD11c⁺ DCs⁴². Similar observations have been made in mouse models of colorectal carcinoma⁴³. In this study, anticancer immune responses required CD11c⁺ DCs to express the nucleic acid-sensing protein cyclic GMP-AMP synthase (cGAS; also known as MB21D1), STING and IFNAR1, and the local injection of recombinant IFN β could restore the therapeutic effects of radiation therapy in cGAS-deficient or STING-deficient mice but not in IFNAR1-deficient mice⁴³. Therefore, IFN β must work downstream of cGAS and STING to stimulate radiotherapy-induced immunosurveillance.

Type I IFN signalling is also essential for the therapeutic effects of several immunotherapies. In mouse models, the graft-versus-leukaemia (GVL) activity of allotransplanted T cells requires them to express IFNAR1 (REF. 44). Imiquimod, which is a synthetic TLR7 and TLR8 agonist that is topically applied to treat skin cancers, promotes the IFNAR1-dependent recruitment of pDCs into the tumour bed. These pDCs produce type I IFNs in a TLR7-dependent manner, which activates an autocrine circuit that promotes tumour killing by pDCs through the production of cytotoxic molecules⁴⁵. Accordingly, the absence of *Tlr7* or *Ifnar1* abolishes the therapeutic activity of imiquimod in melanoma-bearing mice, which correlates with a reduced expression of cytotoxic molecules by tumour-infiltrating pDCs⁴⁵.

Indirect evidence also suggests that type I IFNs might be involved in the therapeutic activity of checkpoint blockers such as the clinically used monoclonal antibodies ipilimumab, which targets cytotoxic T lymphocyte-associated protein 4 (CTLA4), and nivolumab, which targets programmed cell death protein 1 (PD1; encoded by *PDCD1*)⁴⁶. Indeed, the synergistic anticancer effects achieved by the simultaneous blockade of CTLA4 and PD1 ligand 1 (PDL1; also known as CD274) in mice bearing murine B16-SIY melanomas were lost in STING-deficient hosts⁴⁷.

The antineoplastic effects of so-called immunomodulatory drugs (IMiDs), such as the clinically used molecule lenalidomide (which is particularly effective against multiple myeloma), have for a long time been attributed to the ability of these agents to alter cytokine signalling⁴⁸. Among other immunological effects, IMiDs were shown to limit the secretion of tumour necrosis factor (TNF), to promote interleukin-2 (IL-2) synthesis, to target IRF4 for proteasomal degradation and to activate IRF7, thereby stimulating the synthesis of IFN β ^{49,50}. A recent study has suggested that the remarkable therapeutic activity of lenalidomide against multiple myeloma originates from its ability to trigger the degradation of two transcription factors that are required for the survival and the proliferation

of B cells — Ikaros family zinc finger 1 (IKZF1) and IKZF3 (REF. 51). However, whether type I IFN signalling is required for the clinical efficacy of IMiDs has not been formally elucidated.

Although the production of type I IFNs has for a long time been regarded as an obstacle for anticancer virotherapy (because type I IFNs block viral replication), mounting evidence suggests that type I IFNs actively contribute to the induction of TAA-specific immune responses. In mice, the intratumoural injection of the oncolytic Newcastle disease virus combined with systemic CTLA4 blockade can eradicate B16 melanomas through immune responses that require CTLs, NK cells and IFNAR1 (REF. 52). Similarly, the local administration of an IL-12-encoding variant of the Semliki Forest virus elicits tumour-specific CTLs only if the host expresses IFNAR1 (REF. 53).

Taken together, these examples show the broad implications of type I IFN signalling in essential steps of the immune response that ultimately control tumour growth after anticancer therapy.

Clinical indications for type I IFNs

Following an intense wave of clinical investigation that began in the late 1970s (when type I IFNs were purified from the supernatant of human leukocytes exposed to viruses) and culminated in the mid-1980s⁵⁴, IFN α 2a and IFN α 2b — as unmodified recombinant proteins or as their pegylated IFN variants (which have an improved half-life) — have been approved by various regulatory agencies for the treatment of multiple neoplasms⁵⁵. However, over time they have been displaced in many cases by other, comparatively more efficient, therapies (TABLE 1).

Pegylated IFN α 2b has been used for the treatment of resected stage II and III melanoma⁵⁶ and mediates beneficial effects in patients with ulcerated primary tumours and microscopic nodal disease by favouring the influx of DCs and T cells into neoplastic lesions^{57,58}. Individuals with hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC) can also be treated using adjuvant IFN α . In a cohort of such patients, high intratumoural levels of the cytosolic PRR retinoic acid-inducible gene I (RIG-I; also known as DDX58) predicted response to therapy⁵⁹. This suggests that RIG-I may be required for the response of patients with HCC to IFN α . Consistent with this idea, depleting RIG-I from HCC xenografts compromises the antineoplastic effects of IFN α in mice⁵⁹. RIG-I can stimulate type I IFN production, but how RIG-I functions downstream of IFN α in this setting remains to be clarified.

Pegylated IFN α combined with the multikinase inhibitor imatinib can increase the rate of molecular responses among patients with chronic myeloid leukaemia (CML)^{60,61}. Moreover, treatment with pegylated IFN α after imatinib discontinuation causes sustained remission in a majority of patients with CML^{62,63}. This effect has been speculatively attributed to a therapeutic synergy between imatinib and IFN α at the level of malignant cells (because imatinib promotes IFNAR1 expression)⁶⁴, to the IFN α -mediated mobilization of

Table 1 | **Clinical use of type I IFNs for the treatment of virus-unrelated cancers**

Indications	Observations	Refs
On-label		
Chronic myeloid leukaemia	IFN α 2b was the treatment of choice for patients who were not eligible for a bone marrow transplant before the discovery of imatinib	120
Hairy cell leukaemia	IFN α 2a is still used in patients who are not eligible for rituximab-based immunochemotherapy	121
Melanoma	PegIFN α 2b and IFN α 2b prolong relapse-free survival but not overall survival in patients with melanoma who are at high risk for relapse	122
Multiple myeloma	IFN α 2b was used for a long time but is now being progressively replaced by other agents, including IMiDs	123
Non-Hodgkin lymphoma	IFN α 2b improves the activity of chemotherapy and rituximab in patients with follicular lymphoma	124
Proposed		
Acute myeloid leukaemia	PegIFN α 2a mediates direct anticancer effects, increases the immunogenicity of leukaemic cells and stimulates the cytotoxicity of DCs	69
Castration-resistant prostate cancer	IFN α 2b improves the therapeutic effects of docetaxel	75
Chronic lymphocytic leukaemia	In combination with GM-CSF	70
Cutaneous lymphomas	Most patients respond to subcutaneous IFN α	71
Polycythemia vera	PegIFN α 2a is an alternative to 5-HU for cytoreduction	74
Relapsed follicular lymphoma	High rate of response to IFN α , some of which were durable	72
Systemic mastocytosis	Salvage therapy with IFN α may have some benefit	73
Testicular teratoma	Successful treatment of relapsed tumours with IFN α	76

5-HU, 5-hydroxyuracil; DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IMiD, immunomodulatory drug; pegIFN, pegylated IFN.

leukaemic stem cells (which are sensitive to imatinib)⁶⁰ and to the activation of cellular immune responses specific for proteinase 3 (which is a TAA highly expressed by CML cells)^{62,65}. Importantly, patients with CML who successfully discontinued IFN α treatment showed higher proportions of circulating CD4⁺ effector memory T cells with an improved secretory capacity and of CD8⁺ central memory T cells than healthy individuals⁶⁶. Circumstantial evidence also suggests that the positive effect of IFN α on myeloproliferative neoplasms coincides with an increased frequency of circulating CD56^{hi} NK cells⁶⁷ (compared with untreated patients, patients treated with chemotherapy and healthy subjects) and with a high expression level of IFN γ by these lymphocytes⁶⁸. Whether the therapeutic effect of IFN α on other haematological cancers (TABLE 1) also involves immunostimulatory effects remains to be determined.

The safety and efficacy of recombinant IFN α 2a and IFN α 2b, used either as stand-alone immunostimulatory interventions or combined with various therapeutic paradigms, are being globally assessed in more than 100 open clinical studies involving patients affected by a wide range of haematological and solid tumours for which type I IFNs are currently not approved (ClinicalTrials.gov). Promising results have already been obtained in patients with acute myeloid leukaemia⁶⁹, chronic lymphocytic leukaemia⁷⁰, primary cutaneous lymphoma⁷¹, relapsing follicular lymphoma⁷², systemic mastocytosis⁷³,

polycythemia vera⁷⁴, castration-resistant prostate carcinoma⁷⁵ and testicular teratoma⁷⁶ (TABLE 1). It will be interesting to see whether regulatory agencies will extend the approval of IFN α 2a or IFN α 2b to any of these disorders.

Targeted type I IFN-based immunotherapies

The systemic administration of type I IFNs may have paradoxical immunosuppressive effects⁷⁷ and is accompanied by major adverse outcomes, the most common of which are fatigue, anorexia, hepatotoxicity, flu-like symptoms and severe depression⁷⁸. For these reasons, attempts have begun to specifically deliver type I IFNs to the tumour microenvironment (FIG. 2).

One possible means to target type I IFNs to specific cell populations (including malignant cells or specific leukocyte populations) is to conjugate it to, or fuse it with, monoclonal antibodies to generate so-called ‘immunocytokines’ (REF. 79). Such immunotherapeutics have already been shown to have antineoplastic effects in rodent tumour models⁸⁰. For instance, C2-2b-2b, an immunocytokine comprising tetrameric IFN α 2b coupled to hL243 (a humanized monoclonal antibody that is specific for HLA-DR) is effective against human myeloma and lymphoma xenografts⁸¹. Similarly, IFN β fused to cetuximab (a clinically approved EGFR-targeting monoclonal antibody)⁸² limits the growth of mouse EGFR-expressing tumours that failed to respond to unmodified cetuximab⁸⁵. Moreover, fusion proteins comprising IFN α or IFN β

Retinoic acid-inducible gene 1 (RIG-I). A cytosolic sensor that responds to viral double-stranded RNA in the cytosol by inducing type I interferon production.

Imatinib

A multikinase inhibitor initially developed as a specific blocker of BCR–ABL, the chimeric kinase that aetiologically underpins leukaemogenesis in Philadelphia chromosome-bearing cells. As imatinib also inhibits KIT and platelet-derived growth factor receptor- β (PDGFR β ; encoded by *PDGFRB*), it is also used in patients with gastrointestinal stromal tumours that overexpress KIT and some myelodysplastic syndromes associated with *PDGFRB* rearrangements.

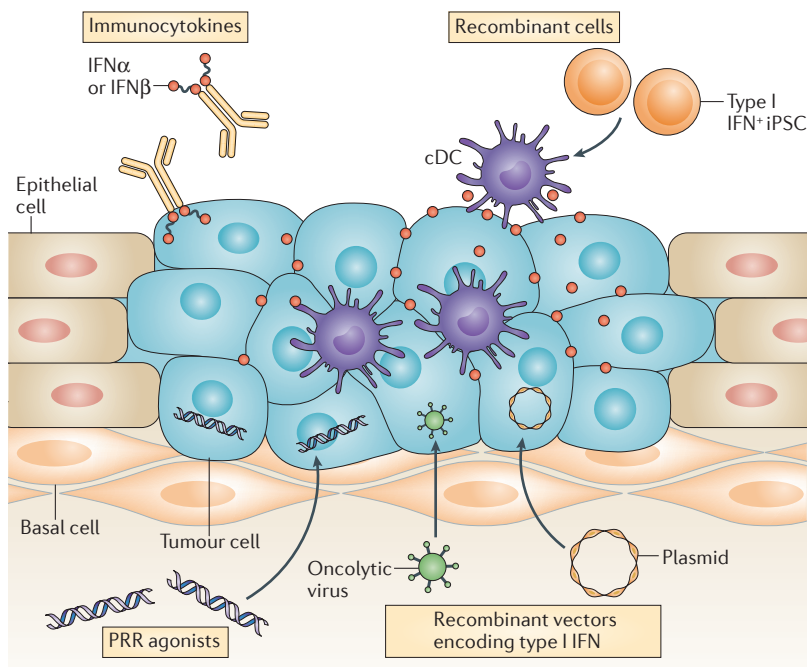


Figure 2 | Experimental targeting of type I IFNs to malignant lesions. Several strategies have been conceived to specifically stimulate type I interferon (IFN) signalling within neoplastic lesions. These include immunocytokines, which are molecules formed of recombinant type I IFNs fused or linked to a tumour-targeting monoclonal antibody or peptide; monocyte-derived conventional dendritic cells (cDCs) or induced pluripotent stem cells (iPSCs) that are genetically modified to express type I IFNs as they mature into cDCs; viral and other tumour-targeting vectors encoding type I IFNs; and pattern recognition receptor (PRR) agonists delivered into the tumour microenvironment.

and an HER2-specific monoclonal antibody are more efficient against HER2-expressing neoplasms than a therapeutic regimen based on the unmodified monoclonal antibody^{35,83}. Of note, the therapeutic effects of type I IFN-containing immunocytokines targeted to EGFR seem to rely on adaptive immune responses involving CTLs (but not B cells or NK cells), CD11c⁺ DCs and IFNAR1 expression by haematopoietic cells (and more specifically by CD11c⁺ DCs). Moreover, the local TAA-specific immune response elicited by this approach correlates with an increase in the numbers of circulating IFN γ -producing CTLs and CD86⁺ DCs³⁵. These encouraging results should stimulate the clinical development of type I IFN-based immunocytokines.

Various cell types can be engineered to express type I IFNs to boost their own antineoplastic activity or to support the tumour-killing ability of immune effector cells from the host. An NK cell line genetically engineered to express human IFN α has improved cytotoxicity functions against HCC cells *in vitro*, as well as in xenograft tumour models⁸⁴. Similarly, mesenchymal stem cells modified to express mouse IFN α potentially halt the growth of B16 melanomas *in vivo*, an effect that was dependent on T cells and NK cells⁸⁵. As an alternative approach, human myeloid cells overexpressing IFN β have been created by transducing induced pluripotent stem cells (iPSCs) with an IFN β -coding lentivirus, followed by the induction of differentiation *in vitro*⁸⁶.

Induced pluripotent stem cells (iPSCs). A type of pluripotent stem cell that can be generated directly from adult mature cells. Once they have been obtained, iPSCs can be differentiated into almost any cell type.

When inoculated into the peritoneal cavity of immunodeficient mice, such IFN β -expressing myeloid cells showed potent antineoplastic effects against NUGC-4 gastric carcinomas⁸⁶. Along similar lines, human haematopoietic stem cells have been genetically modified so that transgenic human IFN α is produced only by differentiated monocytes (but not by their undifferentiated precursors)⁸⁷. The rationale behind this strategy is that the premature expression of IFN α would induce a proliferative arrest in monocyte precursor cells upon the activation of an autocrine circuit. In chimeric mice bearing a human immune system, these cells were capable of eliciting the clearance of transplanted human tumours in a process that involved the reprogramming of the tumour environment towards an immunostimulatory state upon the infiltration of neoplastic lesions by IFN α -secreting macrophages⁸⁷.

Type I IFN-encoding vectors of various types have been directly injected into tumours. For instance, an adenovirus encoding IFN α was shown to reduce the intratumoural abundance of T_{Reg} cells and to promote the accumulation of T helper 17 (T_H17) cells in CT26 colorectal carcinomas evolving in BALB/c mice, probably as a consequence of increased IL-6 production by CD11c⁺ DCs⁸⁸. Moreover, the intratumoural injection of an mRNA encoding IFN β fused to the ectodomain of transforming growth factor- β (TGF β) receptor 2 (TGFBR2) significantly delayed tumour growth by enhancing the ability of TAA-specific CTLs to mediate cytotoxic effects, which was further potentiated by the blockade of PD1 (REF. 89).

Another possible way to induce the production of type I IFNs within neoplastic lesions involves the activation of specific PRRs. In cancer cells, RIG-I can be stimulated by 5'-triphosphate RNA species (ppp-RNA), resulting in the initiation of cell death, as well as in the production of type I IFNs and other factors that promote innate immunity⁹⁰. Thus, the administration of ppp-RNA might mimic a viral infection and might initiate a type I IFN-driven immune response that overcomes tumour-mediated immunosuppression⁹¹. It is feasible to generate ppp-RNAs that not only activate RIG-I but also interfere with the expression of immunosuppressive cytokines such as TGF β 1, which results in superior therapeutic efficacy in preclinical tumour models⁹². Attempts are also underway to generate new CpG oligodeoxynucleotide (ODN) multimers as nano-rings, because these structures potently stimulate IFN α production by pDCs and elicit vigorous anticancer immune responses⁹³. Furthermore, the intratumoural injection of the cGAS agonist 2'3'-GAMP increases T cell-dependent anticancer immune responses triggered by radiotherapy by stimulating the production of type I IFNs via a STING-dependent pathway⁴⁵. Finally, the local administration of polyinosinic-polycytidylic acid (polyI:C) — which is an agonist of TLR3, RIG-I and melanoma differentiation-associated protein 5 (MDA5; also known as IFIH1)⁹⁴ — can stimulate the IFNAR1-dependent recruitment of immune cells to neoplastic lesions, hence inducing therapeutically relevant anticancer responses in preclinical models of melanoma²⁸.

Indoleamine 2,3-dioxygenase 1 (IDO1). An enzyme that catalyses the first and rate-limiting reaction of degradation of the amino acid L-tryptophan. IDO1 mediates robust immunosuppressive effects, not all of which depend on its ability to deplete L-tryptophan and favour the accumulation of L-kynurenine.

Taken together, the aforementioned strategies can promote a selective increase in type I IFN concentration within neoplastic lesions. This drives potent anticancer effects that seem to be mediated by immune effector cells and that avoid the toxicities associated with the systemic administration of type I IFNs.

Conclusions and perspectives

Although it was initially thought that type I IFNs exert direct anticancer effects by activating IFNAR signalling in malignant cells — hence inhibiting cell cycle progression⁹⁵, promoting terminal differentiation⁶⁷, inducing apoptosis⁵⁰ or mobilizing stem cells^{60,96} — it is becoming increasingly clear that type I IFNs mainly function (but perhaps not only) by stimulating anticancer immune responses.

Such an immunostimulatory effect can originate from type I IFNs secreted by malignant cells or by intratumoural DCs. Moreover, it can involve autocrine or paracrine signalling circuits induced by stimulation of IFNARs expressed by malignant, vascular and/or immune cell compartments of the tumour mass. Depending on the experimental model, the antineoplastic activity of exogenously administered type I IFNs has indeed been attributed to IFNAR signalling in immune cells^{35,97,98}, endothelial cells⁹⁹ or malignant cells³⁶. Taken together, these findings suggest that targeting type I IFNs to a specific cellular compartment of the tumour mass may mediate optimal therapeutic effects in some, but not in all, cancers.

Irrespective of this unanswered question, type I IFN signalling within neoplastic lesions seems to be essential for both natural and therapy-induced immunosurveillance, which indicates that the expression levels of these cytokines, as well as of their downstream effectors (for example, ISGs), should be further investigated as prognostic and predictive biomarkers. Therapies designed to increase the intratumoural concentration of type I IFNs can have antineoplastic effects following the induction of anticancer immune responses. Thus, it will also be important to optimize the methods to selectively deliver type I IFNs to the tumour bed in a way that results in superior immunostimulatory effects but that avoids possibly detrimental outcomes, such as inducing the expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1)¹⁰⁰. Moreover, it will be essential to advantageously combine type I IFN (or agents eliciting its production) with other immunostimulatory agents, such as checkpoint blockers^{28,89,101}, granulocyte-macrophage colony-stimulating factor (GM-CSF) or other cytokines^{55,70}, and inhibitors of the transcription factor STAT3, which is involved in multiple immunosuppressive circuits¹⁰². It can be anticipated that strategies for the appropriate stimulation of type I IFN signalling will lead the way to the development of ever-more effective anticancer therapies. By taking advantage of a sophisticated defence system that originally evolved to clear virus-infected cells, tumour immunologists should dedicate substantial efforts to inducing a state that mimics viral infection, featuring the secretion of type I IFNs, in malignant tissues.

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Competing interests statement

The authors declare no competing interests.

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