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Review

Type I interferons in infection and cancer: Unanticipated dynamics with therapeutic implications

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Abstract

If there is a great new hope in the treatment of cancer, the immune system is it. Innate and adaptive immunity either promote or attenuate tumorigenesis and so can have opposing effects on therapeutic outcome.

Originally described as potent antivirals, Type-I-IFNs were quickly recognized as central coordinators of tumor-immune system interactions. Type-I-IFNs are produced by, and act on, both tumor and immune cells being either host-protecting or tumor-promoting. Here, we discuss Type-I-IFNs in infectious and cancer diseases highlighting their dichotomous role and raising the importance to deeply understand the underlying mechanisms so to reshape the way we can exploit Type-I-IFNs therapeutically.

Keywords

IFNs; tumor immunity; anticancer therapy; immunotherapy; cancer stem cells

Abbreviations

AIM2 absent in melanoma 2;

AP-1 activated protein-1;

ATM ataxia-telangiectasia mutated;

CARD caspase activation and recruitment domain;

CARDIF CARD adaptor-inducing IFNβ;

CDKN1A cyclin dependent kinase inhibitor 1A;

cGAMP cyclic guanosine monophosphate–adenosine monophosphate;

cGAS cyclic GMP-AMP synthase;

CSC cancer stem cell;

CSF1 colony stimulating factor 1;

CTL cytotoxic T lymphocyte;

CXCL10 C-X-C motif chemokine ligand 10;

DAI DNA-dependent activator of IRFs;

DAMPs damage-associated molecular patterns;

DC dendritic cell;

DDX DExD/H-box helicases;

EGFR epidermal growth factor receptor;

EMT epithelial-to-mesenchymal transition;

FDA Food and Drug Administration;

FASLG FAS ligand;

HER2 human EGFR 2;

HLA human leucocyte antigen;

HSPC hematopoietic stem/progenitor cell;

ICD immunogenic cell death;

IFI16 IFN-γ-inducible 16;

IFN interferon;

IFNAR IFN-α/β receptor;

IFNGR IFN-γ receptor;

IKKε IkB kinase ε;

IL interleukin;

IPS-1 IFNβ promoter stimulator-1;

IRF IFN regulatory factor;

ISG IFN-stimulated gene;

ISGF3 IFN-stimulated gene factor 3;

JAK Janus kinase;

LGP2 laboratory of genetics and physiology 2;

LPS lipopolysaccharide;

Mal MyD88 adaptor-like;

MAPK14 mitogen-activated protein kinase 14;

MAVS mitochondrial antiviral signalling adaptor;

MCA 3'-methylcholanthrene;

MDA5 melanoma differentiation-associated protein 5;

MDSC myeloid-derived suppressor cells;

MHC-I major histocompatibility complex-I;

MyD88 myeloid differentiation primary response gene 88;

MX1 MX dynamin-like GTPase 1;

NF-κB nuclear factor κB;

NK natural killer;

NLR NOD-like receptor;

NOD2 NOD-containing protein 2;

OAS 2′-5′-oligoadenylate synthetase;

PAMPs pathogen-associated molecular patterns;

pDC plasmacytoid DC;

PD-L1 programmed death–ligand 1;

PKR protein kinase R;

POLR3 RNA polymerase-III;

PRR pathogen recognition receptor;

p53/TP53 tumor protein p53;

RANK receptor activator of NF-κB ligand;

RIG-I retinoic acid-inducible gene-I;

RLR RIG-I-like receptor;

ROS reactive oxygen species;

SARM sterile armadillo-motif-containing protein;

SOCS suppressor of cytokine signalling;

STAT signal transducer and activator of transcription;

STING stimulator of IFN genes;

TAA tumor-associated antigens;

TBK1 TANK-binding kinase 1;

TLR Toll-like receptor;

TME tumor microenvironment;

TMEM173 transmembrane protein 173;

TNF tumor necrosis factor;

TRAIL TNF-related apoptosis-inducing ligand;

TRAM TRIF-related adaptor molecule;

Treg regulatory T cells;

TREX1 three prime repair exonuclease 1;

TRIF TIR-domain containing adaptor protein-inducing IFNβ;

TYK2 tyrosine kinase-2;

VEGF vascular endothelial growth factor;

VISA virus-induced signalling adaptor.

Introduction

The sensing of altered-self, such as changes in tissue/organ homeostasis or integrity, and hence the need to detect and protect against potential danger (*e.g.*, cellular stress, damage, or abnormal death), is upsetting the traditional view of immunity as a response to solely alien microbes and molecules[1]. In particular, it is now clear that cancer cells, either transformed by foreign pathogens (*e.g.*, human papillomavirus, hepatitis-B virus, Epstein–Barr virus, human T-lymphotropic virus-I, hepatitis-C virus, Kaposi's sarcoma herpesvirus, or *Helicobacter pylori*) or totally aseptic, differ antigenically from their normal counterparts and, similar to virus-infected cells, emit danger signals to license the immune system. Such signals, best known as damage-associated molecular patterns (DAMPs), *de facto* favor the establishment of a productive and long-lasting immune response allowing to clear virus-infected cells (because they express virus-encoded proteins) and tumor cells (because they express tumor-associated antigens, TAA). Intriguingly, anti-viral and anti-tumor immune responses share common DAMPs, among which Type-I-interferons (IFNs) emerge as the *primum movens* for the sequential events bridging innate and cognate immunity[2].

IFNs and their receptors are a subset of the class-2 α-helical cytokines that have been found in all vertebrates, although a systemic phylogenetic knowledge is lagging behind. Based on criteria such as their cellular source, their general biologic properties, their gene structure and the receptor through which they signal, IFNs have been categorized into three distinct families: Type-I, Type-II and Type-III. In humans, Type-I-IFNs consist of 13 partially homologous IFN-α cytokines, a single IFN-β and several not yet well characterized single gene products (IFN-ε, IFN-τ, IFN-κ, IFN-ω, IFN-δ and IFN-ζ) all of which are mostly non-glycosylated proteins of

165--200 aminoacids[3]. The reason for the existence of multiple subtypes may be ascribed to differences in tissue-specific expression, the kinetic of production and the *spectrum* of biological activities[4]. Almost all cells in the body can produce Type-I-IFNs following the recognition of molecules, such as foreign and self nucleic-acids, and a minority of other non-nucleic-acids (collectively known as pathogen associated molecular patterns, PAMPs) by the so-called pathogen recognition receptors (PRRs) located in the plasma membrane, cytosol or endosomal compartments[5]. In the canonical Type-I-IFN signalling, Type-I-IFNs bind to a heterodimeric transmembrane receptor termed IFN-α/β receptor (IFNAR), in turn activating the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. This cascade induces the transcription of few hundreds of IFN-stimulated genes (ISGs), which steer the multiple facets of the cellular response[6]. The Type-II-IFN family consists of a single IFN-γ glycosylated protein of 140 aminoacids, which is produced exclusively by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells under immune and inflammatory *stimuli*. IFN-γ signals through the heterodimeric IFN-γ receptor (IFNGR), consisting of IFNGR1 and IFNGR2 and characterized by a JAK1 binding domain and a STAT1 docking site[7]. The Type-III-IFN family consists of the three subtypes IFN-λ1, IFN-λ2, IFN-λ3 [also known as interleukin (IL)-29, IL-28A and IL-28B, respectively] and the newly identified IFN-λ4[8, 9]. Type-III-IFNs are structurally similar to IFN-γ, but functionally identical to IFN-α/β. Only epithelial-like cells and, to a lesser extent, some immune cells respond to IFN-λs. Type-III-IFNs engage a receptor complex composed of the IFN-λR1 (or IL-28AR) and IL-10R2 chains to induce signalling pathways similar to those of Type-I-IFNs[8].

This Review focuses on Type-I-IFNs and how pathogens and danger signals cross-regulate IFNAR signalling to mount immune defences against virus-related and -unrelated diseases such as cancer. We conclude with open questions, future perspectives and implications for new clinical uses of Type-I-IFNs in oncology.

Pathways triggering production of Type-I-IFNs

As reported in the introduction, Type-I-IFNs can be produced by all nucleated cells in the body. The production of Type-I-IFNs is transient and occurs upon stimulation with viral or other xenogeneic or autologous nucleic-acids of an array of transmembrane and cytosolic PRRs (**Figure 1**). Currently identified

PRRs include Toll-like receptors (TLRs), RIG-I-like receptor (RLRs), NOD-like receptors (NLRs) and DNA sensors[10]. Although viral nucleic-acids are the predominant ligands, other molecules, including viral proteins, bacterial lipopolysaccharide (LPS), lipoproteins or endogenous ectopic proteins, can bind PRRs ultimately leading to Type-I-IFN production and innate immune responses[10].

TLRs, the first PRRs identified, are transmembrane receptors either expressed on the cell surface or associated with intracellular vesicles[11]. To date, 10 functional TLRs have been identified in humans, each of them detecting specific PAMPs. Briefly: lipoproteins are recognized by TLR1, TLR2 and TLR6; double-stranded- and single-stranded-RNAs by TLR3, TLR7 and TLR8; LPS by TLR4; flagellin by TLR5; and DNA by TLR9[11]. Although recent evidence suggests that TLR10 could have either immune-stimulatory[12] or immune-suppressive[13] properties, its exact activating ligand(s) and function are not yet known. TLRs signal through five different adaptor molecules: myeloid differentiation primary response gene 88 (MyD88), MyD88 adaptor-like (Mal), TIR-domain containing adaptor protein-inducing IFNβ (TRIF), TRIF-related adaptor molecule (TRAM) and sterile armadillo-motif-containing protein (SARM)[14]. The association with these proteins recruits and activates the IkB kinase ε (IKKε)/TANK-binding kinase 1 (TBK1) complex. This, in turn, is responsible for the phosphorylation and activation of the IFN regulatory factor (IRF)3, nuclear factor (NF)-κB, and activated protein (AP)1, all of them leading to the first-wave of IFN-β production. IFN-β then triggers the autocrine and paracrine expression of a related factor, IRF-7, which is responsible for a positive feed-back loop initiating the synthesis of several IFN-α subtypes as the second-wave of Type-I-IFNs[15].

Among the cytosolic PRRs, RLRs are a family of DExD/H box RNA helicases (DDX) sensing PAMPs within viral RNA. To date, three RLR members have been identified: (1) retinoic acid-inducible gene (RIG)-I; (2) melanoma differentiation-associated protein (MDA)5; and (3) laboratory of genetics and physiology (LGP)2. RIG-I and MDA5 detect a variety of viruses and share a number of structural similarities including their organization into three domains: a tandem caspase activation and recruitment domain (CARD) region to the N-terminal, a central DDX helicase, and a repressor domain to the C-terminal that, in the case of RIG-I, is

involved in autoregulation[16]. Although presenting a similar organization, LGP2 lacks the N-terminal CARD and is currently thought to be a regulator of RIG-I and MDA5 rather than a *bona fide* PRR[17]. Upon binding to double-stranded-RNAs, RLRs directly interact with a downstream molecule named independently by four different groups as mitochondrial antiviral signalling adaptor (MAVS)[18], IFNβ promoter stimulator (IPS)-1[19], virus-induced signalling adaptor (VISA)[20], and CARD adaptor-inducing IFNβ (CARDIF)[21]. As for TLRs, the association with this mitochondrial-resident protein via CARD induces Type-I-IFN production by IKKε/TBK1 complex.

NLRs are cytoplasmic PRRs with a tripartite structure consisting of a variable N-terminal effector domain, a middle nucleotide-binding domain and a C-terminal leucine-rich repeat domain[22]. Among the more than 20 NLRs identified in humans so far[22], only the cytosolic molecular sensor NOD-containing protein 2 (*NOD2*) was clearly shown to recognize single-stranded RNAs leading to Type-I-IFN production through a mechanism dependent on MAVS and IRF3 activation[23]. Other NLRs are mainly described as regulators of the major histocompatibility complex-I (MHC-I)[24], the inflammasome multiprotein complex assembly[25] and regulated cell death pathways (apoptosis, pyroptosis and pyronecrosis[22]). All these functions go beyond their sensing of DAMPs and PAMPs, which instead remains largely unknown.

The first described PRR for DNA, and still the only known endosomal-based DNA sensor, was TLR9[26]. TLR9 is expressed preferentially in plasmacytoid dendritic cells (pDCs) and acts as a potent inducer of IFN-α via a signalling network dependent on MyD88 and IRF7[26]. Moreover, DNA can end-up in the cytosol through several routes (*e.g.*, intracellular pathogens, lysosome-internalized exogenous DNA from dead cells, or endogenous DNA replication debris) where it can be recognized by more than ten cytosolic receptors[27]. The search for cytosolic DNA sensors first led to the identification of the DNA-dependent activator of IRFs (DAI)[28]. When exogenously expressed in L929 murine fibroblasts, DAI increased Type-I-IFN production in a dose-dependent manner following stimulation by both B- and Z-form DNA[28]. Similarly, knockdown of DAI with specific siRNAs impaired Type-I-IFN production in response to cytosolic DNA[28]. RNA polymerase-III (POLR3), the second cytosolic DNA sensor discovered, was reported to use AT-rich and herpesvirus DNA as

a template to produce 5′-triphosphate RNAs, which then induce Type-I-IFNs by activating RIG-I[29]. However, POLR3 could not account for DAI-independent sensing of non-AT-rich DNA suggesting the existence of additional cytosolic DNA sensors. Remarkably, an adaptor molecule referred to as stimulator of IFN genes (STING) was identified as being crucial for recognizing cytoplasmic DNA and inducing innate immune responses to a variety of DNA pathogens even including certain RNA viruses[30]. Nonetheless, despite the wealth of recent information on the mechanisms whereby STING contributes to signal Type-I-IFN induction, the upstream DNA-sensing events remain largely unknown. Recent evidence suggests that cytosolic DNA is perceived by the cyclic GMP-AMP synthase (cGAS), which then becomes catalytically active and generates the second messenger cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). cGAMP in turn binds to STING stimulating its transit from the endoplasmic-reticulum to perinuclear endosomes where it triggers IRF3 activation via TBK1[30, 31]. Of note, STING-dependent Type-I-IFN production can also be activated by single-stranded-DNA resulting from DNA damage or replication stress[32], by mitochondrial DNA released following apoptotic mitochondrial outer membrane permeabilization[33] and possibly by retroelements not properly metabolized by the three prime repair exonuclease (TREX)1[34].

Two essential mediators of distinct DNA-activated innate responses seem to be the PYHIN proteins absent in melanoma (AIM)2 and IFN-γ-inducible (IFI)16[35, 36]. Moreover, the DDX3, DDX41, DHX9, DDX60, DDX1 and DHX36 helicases were recently involved in DNA immune sensing through a pathway dependent on STING and TBK1[37]. In particular, Liu and co-workers found that, in mouse splenic myeloid DCs with limited basal IFI16 expression, DDX41 was the initial sensor of cytoplasmic DNA inducing Type-I-IFNs and the subsequent IFI16 expression, with this latter operating as an amplifier of innate responses[37].

Along with PAMPs and DAMPs, Type-I-IFNs can also be produced in response to rare physiological *stimuli* such as colony stimulating factor (CSF)1[38], receptor activator of nuclear factor κB (NF-κB) ligand (RANK)[39] and estrogens[40]. More recently, an intriguing correlation between Type-I-IFNs and tumor protein p53 (TP53/p53) was reported[41]. In sum, the absence of p53 was associated with extensive DNA hypomethylation, which resulted in a massive transcription of normally silent retroelements and satellite DNA. The subsequent

accumulation of these newly generated double-stranded-RNA species triggered a "suicidal" Type-I-IFN response[41].

Overall, Type-I-IFN production is tightly regulated by major families of heterologous receptors engaged by diverse ligands during infectious and cancerous diseases. Each of the Type-I-IFN subtypes induces a unique and partially overlapping set of ISGs, able to act at different steps of virus and cancer life cycle.

ISGs: a complex net of host defences

Type-I-IFN-mediated innate immune response is hardwired within genomes to provide a robust first-line of host defence and preserve homeostasis. Once secreted by cells, Type-I-IFNs bind to the same ubiquitous heterodimeric IFNAR1-IFNAR2 receptor[42]. The assembly of IFNAR1, Type-I-IFN and IFNAR2 in a 1:1:1 stoichiometry seems to occur via a two-step process whereby Type-I-IFN first binds to one IFNAR and then promotes the recruitment of the second IFNAR without identified interactions between the two IFNARs[42]. Once assembled, this ternary complex promotes the phosphorylation and activation of IFNAR1-associated tyrosine kinase (TYK)2 and IFNAR2-associated JAK1, which, in turn, phosphorylate cytosolic STAT1 and STAT2. This results in the formation of STAT1-STAT2 heterodimers that dissociate from receptors and migrate into the nucleus where they bind IRF9 to form the heterotrimeric transcriptional complex IFN-stimulated gene factor (ISGF)3. In the final step, ISGF3 binds to specific DNA response-elements transactivating hundreds of ISGs[6]. The nature and precise mechanisms through which ISGs prime cells for enhanced pathogen/danger detection and clearance, and then allow them to recover to normal function are not entirely elucidated. Recent evidence, reviewed in ref. 4, showed that Type-I-IFNs lead to cell-type and context-dependent patterns of ISG expression through a complex modulation of all seven STAT family members and other kinases (*e.g.*, PI3K, p38, ERK, and JNK) in addition to JAK. This may explain the complexity to regulate the pattern and magnitude of so many different biological functions in so many different cells during infection, cancer and inflammation[4]. For more insights in these issues refer to databases on signalling pathways and immune cell types such as Interferome (Interferome.org), Innate DB

(http://www.innatedb.com) and the NIAIDs Systems Biology (http:/www.niaid.nih.gov/labsans resources/labs/about-labs/lsb/Pages/).

Similar to most cytokines, Type-I-IFN cascade is tightly regulated by positive and negative feed-forward and feed-back loops, which collectively ensure that the strength and duration of the response are effective yet limited, thereby preventing the toxic consequences of excessive/prolonged signalling[43]. This balance is finely tuned by host factors operating at multiple levels, including signalling, transcription and translation. To give an example, many components of upstream PRR pathways (including receptors and IRFs) are ISGs[44]. Type-I-IFNs are also reported to induce a network of inhibitors of their own signalling, such as members of the suppressor of cytokine signalling (SOCS) protein family[45]. Overall, a complex net of signalling pathways makes proper use of the Type-I-IFN-ISG system to induce host protection while limiting tissue damage and preventing responses to self. Accumulating evidence indeed suggests that an aberrant activation of immunity by high levels of Type-I-IFNs contributes to the development of autoimmune diseases, such as systemic lupus erythematosus[46]. This observation highlights the importance of understanding the mechanisms maintaining strict control over Type-I-IFN signalling to support the development of smart therapies that eradicate the danger and alleviate autoimmune diseases.

Type-I-IFNs in cancer

Type-I-IFNs are back in the oncological spotlight due to a greater understanding of their role in tumor generation, pathogenesis and treatment. Regardless of their source in the tumor microenvironment (TME), Type-I-IFNs have the potential to exert their opposed anti- and pro-tumorigenic actions acting directly on tumor cells and indirectly on immune infiltrating cells (**Figure 2**).

Cancer-intrinsic effects of Type-I-IFNs

The cancer cell-intrinsic effectiveness of Type-I-IFNs is well documented in experimental animal systems and is reported to depend on specific cellular effects such as growth inhibition[47], modulation of apoptosis[48], differentiation[49], migration[49], alteration of cell surface expression of TAAs[50] and promotion of the epithelial-to-mesenchymal transition (EMT)[51]. Type-I-IFNs are known to affect different phases of the mitotic cell-

cycle (panel 1, **Figure 2**) with the most common perturbation being the G_1 arrest[51]. In a seminal work, Balkwill *et al*. showed that *in vitro* treatment of human breast cancer cell lines with exogenous crude preparations of Type-I-IFNs had a direct anti-proliferative effect that was attributed to the prolongation of the cell-cycle[52]. Accordingly, observations from two independent studies showed that IFN-α inhibited the growth of human prostatic cancer cells and murine macrophages stalling the G_1-S transition through the increased expression of the cyclin dependent kinase inhibitor (CDKN)1A, best known as p21[53, 54]. Type-I-IFNs are also reported to induce other CDK inhibitors, including CDKN1B and CDKN2B (best known as p27 and p15, respectively), whose upregulation leads to cell-cycle blockade at the G_1 phase[55]. More recently, Katayama and colleagues provided evidence that, in human colon cancer cells, the anti-proliferative action of Type-I-IFNs relied on a p21-dependent prolongation of the S phase rather than block in G_1[56]. Yet other nets involved in Type-I-IFN-induced cell-cycle arrest are believed to include the downregulation of the transcription factor MYC and the activation of mitogen-activated protein kinase (MAPK)14 or CRK[57, 58]. Contrasting experimental findings indicate that Type-I-IFNs can either induce tumor cell death[59] or protect cancer cells from chemical-induced apoptosis[60] (panel 2 and 5, **Figure 2**). This discrepancy may be ascribed to the degree of cellular differentiation, tumor-related factors and differences in the TME. Indeed, the administration of Type-I-IFNs was reported to modulate the two major apoptotic responses: the extrinsic or death receptor-mediated pathway and the intrinsic or mitochondrial pathway[48]. Briefly, the former cascade requires ligation of cell-surface death receptors, such as the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in order to activate the initiator caspase-8, whereas the latter requires the release of apoptotic factors such as cytochrome-*c*1 from the mitochondria to activate other cytoplasmic initiator caspases. The ISGs involved in apoptosis include (but are not limited to) FAS, FAS ligand (FASLG), protein kinase R (PKR) and oligoadenylate synthetase (OAS), particularly the 9-2 isozyme (extensively reviewed in ref. 61).

The *in vitro* modulation of cultured tumor cells by Type-I-IFNs has been documented. Some early reports showed that IFN-β has the ability to boost human leucocyte antigen (HLA)-class-I expression[62] (panel 3, **Figure 2**) and modulate the antigenic landscape of cultured melanoma cells[63] (panel 4, **Figure 2**). More

recently, these discoveries were characterized by Dunn *et al.*, who showed that IFN-β simultaneously augments TAA (*e.g.*, Melan-A/MART-1, gp100, and MAGE-A1) and HLA-class-I thus increasing the likelihood of improved immune recognition and cytotoxic killing of tumor targets, respectively[64].

The EMT is a process by which epithelial cells lose their polarization and cell-cell contacts and undergo remarkable morphologic changes switching from an epithelial cobblestone phenotype to an elongated fibroblastic phenotype[65]. The EMT provides for the evolution of cancer cells to the metastatic phenotype and contributes to their invasiveness, stemness and drug resistance[65]. In a recent study, the IFN-α-inducible protein-27 was associated with the EMT marker vimentin in ovarian cancer[66] (panel 6, **Figure 2**). This phenomenon finally led to chemoresistant cells with a cancer stem cell (CSC) phenotype[66]. CSCs are defined as the reservoir of a chemoresistant niche within the tumor and the driving force for tumor relapse[67, 68]. Mounting observations indicate a potential contribution of Type-I-IFN signalling in the generation and/or maintenance of CSCs (panel 7, **Figure 2**). Indeed, IFN-α was reported to affect the migration and invasion of pancreatic ductal adenocarcinoma cells through the upregulation of specific CSC markers such as CD24, CD44 and CD133[69]. In addition, it was recently shown that TLR3 stimulation on somatic cells caused global changes in the expression of epigenetic modifiers leading to enhanced chromatin remodelling, nuclear reprogramming, cell plasticity, pluripotentiality, transdifferentiation and even malignant transformation[70]. In line with these data, experiments in breast cancer cells put in evidence that NF-κB and β-catenin signalling downstream of TLR3 promoted the enrichment of a subset of cells with CSC phenotype[71]. Similarly, in the hematopoietic stem/progenitor cell (HSPC) compartment, chronic Type-I-IFN stimulation resulted in HSPC loss of quiescence and dysfunction[72]. This phenomenon was mainly due to Type-I-IFN-induced accumulation of reactive oxygen species (ROS)[73]. Additional indirect proofs of the tumor growth promoting role of Type-I-IFNs come from recent studies showing that, in cancer cells, Type-I-IFNs upregulated the ISG programmed death-ligand (PD-L)1[74] (panel 8, **Figure 2**). PD-L1 is a cell-surface molecule expressed by most tumor cells that mediates inhibitory signals towards CTLs and thus plays a major role in cancer immune-evasion through CTL exhaustion[75]. It is tempting to speculate that sustained therapeutic responses could rely on the

combination of Type-I-IFNs or Type-I-IFN-inducing therapies with antibodies targeting the PD1–PD-L1 axis. Accordingly, a recent study from Shen *et al.* demonstrated that the oncolytic vesicular stomatitis virus engineered to constitutively express IFN-β had significant anti-leukaemia activity, which was further enhanced when combined with an anti–PD-L1 antibody[76]. These observations lend further support to the double-edge sword of Type-I-IFNs in controlling tumor growth and promoting tumor escape. Further insights are needed to decipher the mechanisms through which Type-I-IFNs may paradoxically favor tumor progression. This will certainly have a great impact in the clinical use of Type-I-IFNs.

Cancer-extrinsic effects of Type-I-IFNs

In addition to the direct impact on cancer cells, Type-I-IFNs have extrinsic effects on tumors regulating processes such as angiogenesis and immunity[77]. Type I IFNs have been long recognized as powerful angiogenesis inhibitors. The effects of Type I IFNs on the vasculature have been mainly attributed to the downregulation of vascular endothelial growth factor (*VEGF*) expression as well as to the impairment of endothelial cell proliferation and migration[78] (panel 9, **Figure 2**). Seminal experimental findings from Schreiber's group strongly suggest that, although the immune system plays a major part in restraining the development of cancer, it may also promote the emergence of tumors that escape immune control[79]. According to the immune-editing model, malignant cells, initially held in check by immune-surveillance means, can grow into clinically manifest tumors provided that (1) they lose the cancer molecular determinants that make them recognizable by immune-effectors (immune-selection) or (2) they actively counteract immune responses (immune-suppression)[79]. Immuno-editing consists of three phases: first, at an early stage malignant cells are recognized and eradicated by immune-effector cells (elimination); second, at a later stage small tumors are still held in check by increasingly less proficient immune responses (equilibrium); and finally, neoplastic cells lose their antigenic properties or establish potent immune-suppressive networks, thus avoiding any control (escape)[79]. Most noteworthy, Dunn *et al* proved that Type-I-IFNs intervene in all these three phases[80]. They demonstrated that endogenously produced Type-I-IFNs were required, in immunocompetent mice, to reject highly immunogenic 3'-methylcholanthrene (MCA)-induced sarcomas and

to prevent the outgrowth of primary carcinogen-induced tumors. Furthermore, they observed that several MCA-induced sarcomas from *Ifnar1*$^{-/-}$ mice were rejected in a T cell-dependent manner in wild-type mice, which suggests that tumors arising in the absence of Type-I-IFN responsiveness are more immunogenic than tumors growing in IFNAR competent hosts[80].

The earliest indication that Type-I-IFNs could stimulate extrinsic antitumor effects was reported in a mouse model of lymphocytic leukaemia, in which it was shown that survival rates were increased by administering crude (mixed-type) IFN preparations, irrespective of whether tumor cells themselves were intrinsically sensitive to the anti-proliferative actions of these IFN preparations[81]. From then, an impressive number of instrumental studies in both mice and humans confirmed the plethora of mechanisms by which Type-I-IFNs act on immune cells to mount a strong antitumor response. In the early 1990s, Ferrantini and colleagues showed that highly metastatic Friend leukemia cells genetically modified to secrete IFN-α1 exhibited a marked loss of their tumorigenic potential when injected into syngeneic immunocompetent mice[82], and inhibited the growth of metastatic parental cells in transplantation assays mainly through $CD8^+$ CTLs[83]. Despite these encouraging data, the clinical development of Type-I-IFNs remained underappreciated for many years. In the past two decades the findings that IFN-α induced the differentiation/activation of DCs (panel 10, **Figure 2**) in both mice[84] and humans[85] have spurred the ideation of new immunotherapeutic regimens. Today, new attention is given to Type-I-IFNs as crucial factors bridging innate and adaptive immunity. Several studies support the importance of Type-I-IFNs as a *stimulus* for the production of various cytokines (*e.g.*, TNF, IL-1, IL-6, IL-8, IL-12, and IL-18) by macrophages[86] (panel 11, **Figure 2**), and as factors that markedly affect DC-mediated TAA retention and cross-priming[87] (panel 12, **Figure 2**) and stimulate antibody-dependent cellular cytotoxicity on established B16 murine melanoma liver micrometastases[88]. Furthermore, Type-I-IFNs were reported to play a major role in the development and differentiation of the Th1 subset, as well as in the generation, activity, expansion and long-term survival of CTLs[89] (panel 13, **Figure 2**). Type-I-IFNs are also responsible for the activation of tumoricidal NK cells (panel 14, **Figure 2**), which represent one of the host key mechanisms to preempt tumor growth[90]. More

recently, the role of Type-I-IFNs in the immunometabolism – which is an emerging field that investigates the interplay between immunological and metabolic processes[91] - gained increasing appreciation (panel 15, **Figure 2**). A substantial number of evidence indicates that signalling downstream of PRRs induces changes in core metabolism of DCs and macrophages, which are crucial in shaping their function and fate[91]. In macrophages, Type-I-IFNs downstream of TLR3 induced a shift in the balance of lipid metabolism away from *de novo* cholesterol and fatty-acid synthesis in favor of the uptake of exogenous lipids[92]. This immunometabolic circuit is critical for host immune responses. In line with this discovery, TLR9 stimulation in pDCs led to an autocrine IFNAR signalling resulting in an increased fatty-acids oxidation and oxidative phosphorylation, which is key for pDC immune functions[93]. Accordingly, fasting or the administration of caloric restriction mimetics has been shown to improve the efficacy of immunogenic chemotherapy correlating with the depletion of immunosuppressive regulatory T (Treg) cells from the TME[94]. Notably, Type-I-IFNs are known to negatively regulate the proliferation and activity of immune-suppressive cells such as Treg cells (panel 16, **Figure 2**) and myeloid-derived suppressor cells (MDSCs; panel 17, **Figure 2**)[77]. Undoubtedly, understanding the multilevel interactions between metabolic, immunologic and Type-I-IFN nets will offer additional tools to manage beneficial and detrimental Type-I-IFN immune effects and reshape the way Type-I-IFN-IFNAR axis can be exploited therapeutically during infection and cancer.

The role of Type-I-IFNs in anticancer therapy

Although soon after their discovery the antiviral activity of Type-I-IFNs attracted widest interest, the first US Food and Drug Administration (FDA) approval for IFN-α2, in 1986, was for cancer treatment (**Figure 3**). Even before recombinant IFNs were available, reduction of disease morbidities with partially purified IFN-α was reported in several studies performed in patients with hairy-cell leukaemia and chronic myelogenous leukaemia[95, 96]. In both cases, however, over time more effective therapeutic regimens than IFN have been devised (*e.g.*, the targeted inhibitor of the activated BCR-ABL tyrosine kinase Imatinib[97]). In following clinical studies, the therapeutic effectiveness of IFN-α2, either as unmodified recombinant proteins or pegylated variants, in inducing at least partial disease regression was reported for other hematological and

solid tumors including myelomas, lymphomas, melanomas, Kaposi's sarcoma, and renal-cell and bladder carcinoma[98]. To date, IFN-α2 is still commonly employed combined with IL-2 in immunotherapeutic regimens for metastatic renal-cell carcinomas and cutaneous melanoma[99, 100]. In addition, more than 100 clinical trials are currently underway worldwide using IFN-α2 as monotherapy or in combination regimens for both hematological and solid malignancies (for further details please refer to ClinicalTrials.gov and ref. 101).

A wide range of conventional chemotherapy, radiotherapy and immunotherapy, including oncolytic virotherapy, currently licensed for use in humans, are particularly successful if they induce tumor-targeting immune responses[102, 103]. The current view is that therapeutic agents must induce a sort of 'viral mimicry', *i.e.*, a combination of stress signals that are usually linked to viral infection such as Type-I-IFNs and are believed to contribute to their clinical effectiveness. We recently showed that Type-I-IFNs lie at the *nexus* that controls immunogenic cell death (ICD) and constitutes a hallmark of successful chemotherapy[2]. In particular, we showed that the treatment of various tumor types (*e.g.*, MCA205-fibrosarcomas and AT3-breast carcinoma) with anthracyclines or oxaliplatin gave rise to the rapid production of Type-I-IFNs, thus mimicking the immune reactions evoked by viruses. We also elucidated the mechanism of Type-I-IFN-mediated ICD demonstrating that (1) hit dying cancer cells emit self nucleic-acids (especially single-stranded RNAs) in the TME, which are sensed by TLR3 on surrounding yet viable cells; and (2) released Type-I-IFNs act as the *primum movens* for the sequential events bridging innate and cognate antiviral immunity through a specific ISG signature that includes soluble chemotactic mediators such as the C-X-C motif chemokine ligand (CXCL)10. This is crucial for the recruitment, selection and differentiation/maturation of engulfing cells thus dictating the immunogenic outcome of cell death. Corroborating this evidence, the efficacy of anthracyclines was lost upon co-administration of anti-IFNAR or anti-IFN-α/β neutralizing antibodies[2]. Importantly, in breast cancer patients, increased expression levels of the ISG MX dynamin-like GTPase (MX)1 predicted the likelihood of response to anthracycline-based treatment in neoadjuvant and adjuvant settings[2]. In previous studies, Type-I-IFNs were described as crucial mediators of the off-target immunomodulatory effects of

cyclophosphamide, an alkylating agent inducing ICD[104] responsible for the expansion of memory $CD4^+$ and $CD8^+$ T cells[105] as well as of DCs[104]. In patients with hematological malignancies, the administration of high-dose cyclophosphamide induced a rapid, transient and broad transcriptional modulation on peripheral blood mononuclear cells resulting in DNA damage, cell death and, noticeably, a Type-I-IFN signature[106]. This promoted the establishment of a systemic sterile inflammatory response characterized by the release of endogenous adjuvant signals able to enhance the efficacy of immunotherapy[106]. Similar to chemotherapy, radiation therapy was also reported to increase the levels of Type-I-IFNs and CXCL10 in the TME[107]. In one of these studies, CXCL10 was shown to promote tumor $CD8^+$ T cell-homing and cytolytic activity[107]. Subsequent observations revealed that radiation-mediated antitumor immunity in immunogenic tumors requires a functional cytosolic DNA-sensing pathway upstream of Type-I-IFNs[108]. Accordingly, Hartlova and colleagues recently found that in the absence of ataxia-telangiectasia mutated (ATM, which is an apical component of the DNA damage response) the accumulation of DNA lesions generated spontaneously or provoked by irradiation induced Type-I-IFNs by STING-mediated signalling[32]. Type-I-IFNs in turn primed the innate immune system for a rapid and amplified response to microbial and environmental threats. In addition, Type-I-IFNs boosted the antineoplastic activity of antibodies specific for oncogenic receptors, such as epidermal growth factor receptor (EGFR) and human EGFR (HER)2, mobilizing DCs to cross-present TAA to CTLs[74]. However, despite these observations strongly support the antitumor and immune-stimulatory effects of Type-I-IFNs, paradoxical proofs of a dichotomous, detrimental tumor growth-promoting role for these cytokines are also reported. In this context, some harmful effects seem to depend on the ability to induce immune-checkpoint pathways as a major mechanism of immune-resistance, particularly against CTLs specific for TAAs. As reported above, Type-I-IFNs upregulate PD-L1 in tumor cells[2, 74], which can lead to T cell exhaustion[109]. It remains a central goal of studies on tumor immunity to elucidate the multitude of molecular nets activated by Type-I-IFNs. Big issues to solve are when and through which pathways Type-I-IFNs counteract or promote tumor growth. These insights will likely pave the way to more effective IFN-based immunotherapies.

Conclusions and perspectives

Type-I-IFNs are among the most pleiotropic cytokines and are produced and sensed by almost every cell type in the body. The discovery of Type-I-IFN role in cancer immune-surveillance at first, and cancer immune-editing later, made these cytokines and the immune sensing networks that drive their production very attractive for deeper investigation in preclinical and clinical contexts. As cancer-related genomic information is constantly published, it is emerging that Type-I-IFNs can be produced by, and act on, both malignant and immune cells, thus eliciting immune responses via tumor cell-intrinsic or extrinsic means. Type-I-IFNs, either naturally produced, exogenously administered or induced by chemotherapy, radiotherapy or oncolytic virotherapy exert all biological effects through the action of ISGs. Therefore, efforts to decipher the specific functions of individual ISGs on the reciprocal crosstalk between cancer cells and immune cells may likely help to fulfil IFN therapeutic efficacy and identify predictive biomarkers of response. Taken into account the dual role of Type-I-IFNs in containing and favoring tumor growth, it will be important to understand which subtype of, at which time point and through which mechanisms Type-I-IFNs cease to be immune-effectors and flip to become immune-suppressors and CSC-promoters. The limited efficacy of Type-I-IFNs in cancer medicine may likely reflect this gap of knowledge.

Matter-of-factly, Type-I-IFNs have more than reached the potential envisioned by early discovering virologists, however answering these questions will certainly have a tremendous impact on tumor immunology and biomedicine.

Disclosure of interest

The authors declare no conflicts of interest.

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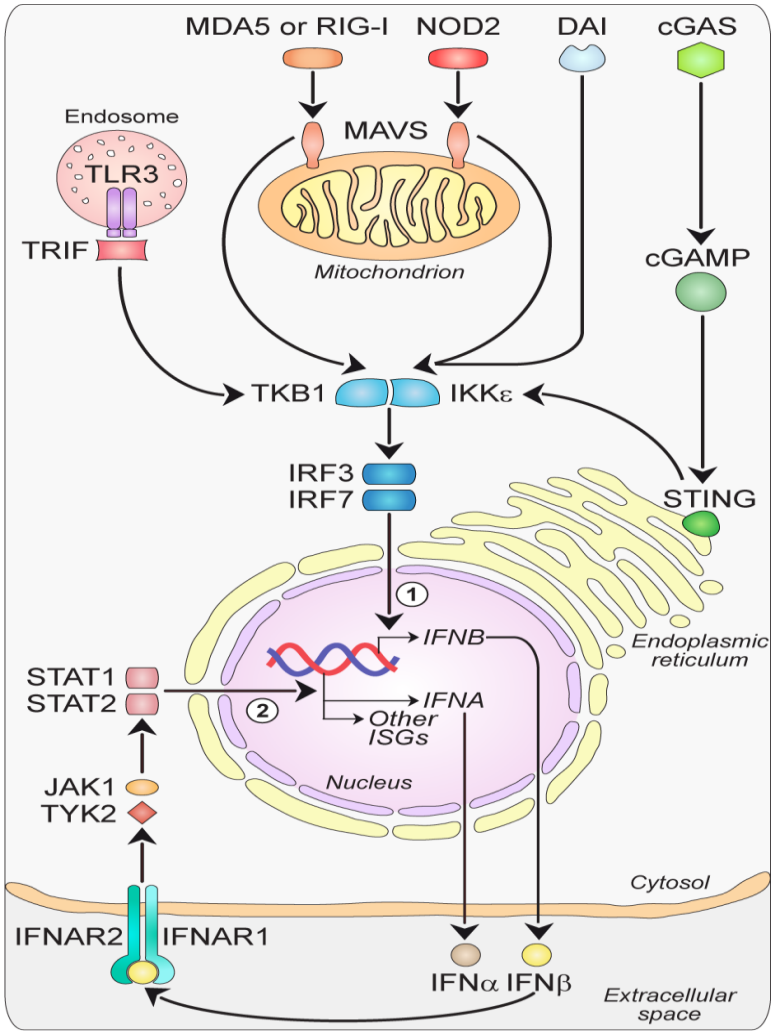

Figure 1

Figure 1: Major intracellular pathways leading to Type-I-IFN production. Families of sensors, known as PRRs, are available in the cells to detect viral and danger products, and induce the expression of Type-I-IFNs. One set of PRRs is localized in endosomal vesicles, while another set senses components in the cytoplasm. The endosome-associated TLR3 and the cytosolic MDA5, RIG-I and NOD2 sense double-stranded and single-stranded RNAs through the activation of adaptor molecules such as TRIF and MAVS, respectively. TRIF and MAVS in turn converge to activate the TBK1-IKKε kinase complex. This culminates in the activation of the transcription factors IRF3 and IRF7, which translocate to the nucleus and participate in the induction of a first wave of IFN-β production (1). IFN-β in turn acts in an autocrine/paracrine manner binding to the heterodimeric receptor IFNAR1-IFNAR2. This is followed by the activation of a JAK-STAT signalling pathway leading to a second wave of IFN-α production as well as to the transcription of other antiviral genes

(2). Other PRRs sensing DNA are DAI and cGAS, with this last catalysing the formation of ligands for STING, upstream of the TBK1-IKKε complex, which finally drives the expression of *IFNA* and *IFNB*. cGAS: cyclic GMP-AMP synthase; DAI: DNA-dependent activator of IRFs; IFNs: interferons; IFNAR: IFN-α/β receptor; IKKε: IkB kinase ε; IRF: IFN regulatory factor; ISG: IFN-stimulated genes; JAK: Janus kinase; MAVS: mitochondrial antiviral signalling adaptor; MDA5: melanoma differentiation-associated protein 5; NOD2: nucleotide oligomerization domain 2; PRRs: pathogen recognition receptors; RIG-I: retinoic acid-inducible gene-I; STAT: signal transducer and activator of transcription; STING: stimulator of IFN genes; TBK1: TANK-binding kinase 1; TLR3: Toll-like receptor 3; TRIF: TIR-domain containing adaptor protein-inducing IFNβ.

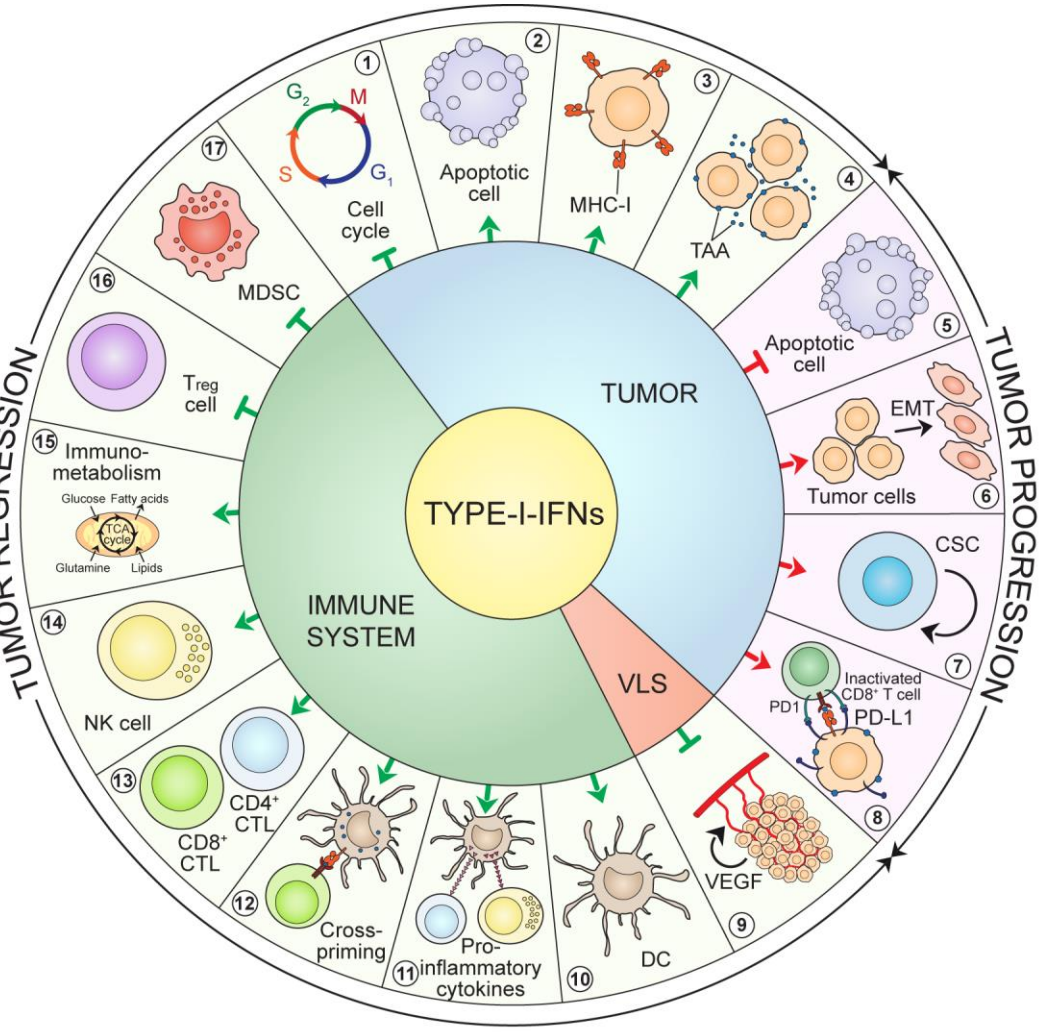

Figure 2

Figure 2: Type-I-IFN-triggered signals. Type-I-IFNs may favor tumor regression and/or tumor progression by acting on tumor cells, immune cells and endothelial cells via various mechanisms. First, acting on tumor cells Type-I-IFNs may promote either tumor regression, by inducing cell-cycle arrest (1), apoptosis (2) and enhanced immunogenicity through cell surface expression of MHC-I (3) and TAAs (4), or tumor progression by inducing resistance to apoptosis (5), EMT (6), tumor-cell stemness (7), and the upregulation of immune-inhibitory signals such as PD-L1 (8). Second, acting on the vascular and lymphatic system Type-I-IFNs inhibit angiogenesis through VEGF downregulation (9). Finally, acting on the immune system Type-I-IFNs stimulate the maturation of DCs (10), promote the release of pro-inflammatory cytokines (11), favor CTL cross-priming (12), foster the activation and survival of $CD8^+$ and $CD4^+$ T cells (13) and of NK cells (14), have a crucial role on core energetic metabolism regulation (15), and negatively regulate immune suppressive

Treg cells (16) and MDSCs (17). CSC: cancer stem cell; CTL, cytotoxic T lymphocyte; DC: dendritic cell; EMT: epithelial-to-mesenchymal transition; IFNs: interferons; MDSCs: myeloid-derived suppressor cells; MHC-I: major histocompatibility complex-I; NK: natural killer; PD1: programmed death 1; PD-L1: programmed death–ligand 1; TAAs: tumor-associated antigens; TCA: tricarboxylic acid; Treg: regulatory T cells; VEGF: vascular endothelial growth factor; VLS: vascular and lymphatic system.

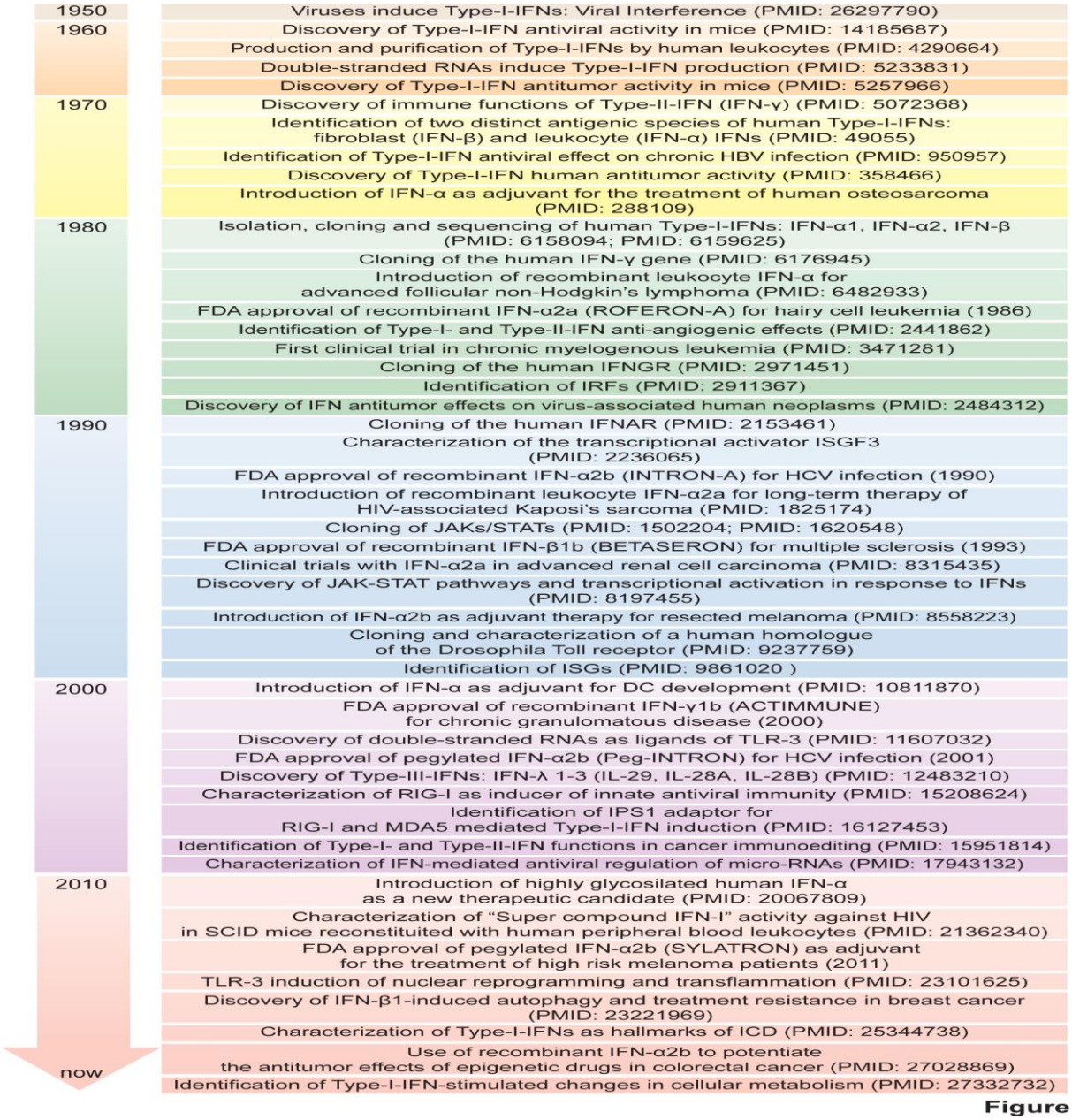

Figure 3: Timeline of IFN discovery and clinical use. The discovery of IFNs evolved from studies of viral interference beginning in 1950. Since then, great attention has been devoted to the molecular understanding and clinical use of IFNs for virus-related and unrelated malignancies. DC: dendritic cell; FDA: Food and Drug Administration; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; ICD: immunogenic cell death; IFNs: interferons; IFNAR: IFN-α/β receptor; IFNGR: IFN-γ receptor; IRF: IFN regulatory factor; ISG: IFN-stimulated gene; ISGF3: IFN-stimulated gene factor 3; JAK: Janus kinase; MDA5: melanoma differentiation-associated protein 5; RIG-I: retinoic acid-inducible gene-I; SCID: severe

combined immunodeficiency; STAT: signal transducer and activator of transcription; TLR3: Toll-like receptor 3.