Interferon-Based Biopharmaceuticals

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Interferons are secreted autocrine and paracrine proteins, which by regulating several biochemical pathways have a spectrum of clinical effectiveness against viral infections, malignant diseases, and multiple sclerosis.

Interferon biopharmaceutical recombinant DNA production purification bioprocess development formulation excipient drug delivery system route of administration

1. Clinical Importance of Interferon-Based Biopharmaceuticals and Market Overview

The lack of effective therapies for the treatment of a variety of human diseases has caused numerous health issues [1], representing the major driving force of Research and Development (R&D) activities toward the development of innovative medicines. In this regard, the emergence of biopharmaceuticals has allowed tremendous improvements in life quality [2], being at the cornerstone of the progress achieved in the last decades on the prevention and treatment of a wide range of diseases (e.g., cancer, infectious diseases, neurodegenerative diseases, among others). Biopharmaceuticals, also called biotherapeutics or biologicals, are products of biological origin such as proteins, nucleic acids, blood-derived products, somatic cells, or derivatives that are produced or extracted from living sources (e.g., microorganisms, cells, plants, or animals) [3][4][5]. Nowadays, recombinant therapeutic proteins and antibodies are considered the most abundant types of biopharmaceutical products in the market [3]. The success of biopharmaceutical-based therapies is linked to the development of recombinant DNA technology in late 1970s, which has allowed the large-scale production of human proteins and strongly stimulated systematic clinical investigations using new therapeutic approaches [6]. Following the approval of the first biopharmaceutical-insulin-in 1982, this market has been rapidly growing. According to the literature, from 2015 to 2018, approximately 112 biopharmaceuticals were approved in the United States of America (USA) and in the European Union (EU), essentially doubling the typical five-yearly historical approval pace and thus demonstrating the high demand for such products [3]. The overall growth of the biopharmaceutical market occurs due to two factors: the first one is related to the increment in the use of this type of product, and the second is closely related to the appearance of biosimilars [3][7][8]. Biosimilars are biological products similar to already existing medicines whose patents have expired [7][8], entering into the market with lower costs while exhibiting the same effects (quality, safety, and effectiveness) as the original biopharmaceutical [7]. Moreover, the global sales of therapeutic

proteins have been increasing, being forecasted to increase on the approval of this type of therapeutic biomolecule in the coming years [3][7].

Among therapeutic proteins, the role of interferons (IFN) should be underlined, as they have been marketed for over 30 years with a considerable impact on the global therapeutic proteins market [3]. However, as recently highlighted by Timmerman [9] on the history of interferon's trajectory, from the viral interference to the Hoffmann-La Roche product (Roferon A®, Hoffmann-La Roche, Basel, Switzerland), a series of obstacles had to be overcome-namely, restrictions to working with recombinant DNA, -to be in line with the interests of commercial partners and their demands for patent protection while addressing the desire by academic researchers working in the field for scientific outputs. IFN sales peaked between the 1980s and 2000s, as they were abundantly marketed and classified as "multiple drugs", with an increasing range of therapeutic effects.

In a period of just six years, from 1986 to 1992, the world IFN market increased by approximately $740 million [10]. More recently, the global IFN market was valued at $6.9 billion in 2019, and it was estimated that it could grow to about $7.5 billion by 2020 due to an increasing demand for the use of IFNs along with antiretrovirals and antimalarial drugs in the treatment of SARS-CoV-2 disease (COVID-19) patients [11]. Furthermore, these projections are supported by the increasing incidence of chronic diseases, such as hepatitis B, hepatitis C, and multiple sclerosis, coupled with the use of IFNs in combinatorial therapies, the increasing adoption of IFN biosimilars with possible prophylactic or therapeutic effectiveness against virus pandemics, the advent of novel drug delivery systems, and continuous R&D activities involving IFNs [11]. Due to their relevance, several IFN products are currently in different stages of clinical trials. By January 2021, 172 active clinical trials involving the application of therapeutic IFN-based products were at different stages of development: 2 are in early phase 1, 50 in phase 1, 70 in phase 2, 28 in phase 3, and 6 in phase 4 of clinical trials [12].

The different clinical applications of IFNs and their corresponding marketed biological medicines are summarized in Table 1 [3][13][14]. Several IFN subtypes are well established in the market for the treatment of several pathologies, mainly in oncological treatment, as well as multiple sclerosis and chronic hepatitis C. To date, 21 formulations for the administration of IFN have received approval from EU and USA regulatory agencies, of which five have been withdrawn from the market—Infergen® (Three Rivers Pharmaceuticals, Warrendale, PA, USA) in 2006 (EU), Roferon A® (Hoffmann-La Roche, Basel, Switzerland) in 2007, Viraferon® (Schering-Plough Corporation, Brussels, Belgium) in 2008 (EU), Albinterferon®/Albuferon® in 2010 (Novartis, Basel, Switzerland; Human Genome Sciences, Rockville, MD, USA), and ViraferonPeg® (Merck Sharp & Dohme Corp., Kenilworth, NJ, USA) in 2021 (EU). Rather than safety and efficacy issues, these products have been generally withdrawn from market due to requests of marketing authorization holders and the availability of similar products in market.

Table 1. Therapeutic interferons approved in the United States of America (USA) and European Union (EU).

Interferon (IFN) Type/Subtype	Clinical Indication	Commercial Name	Active Pharmaceutical Ingredient	Approval Date
IFNα (I)	Hairy cell leukemia; AIDS-related Kaposi's sarcoma; Chronic myelogenous leukemia; Cutaneous T-cell lymphoma; Chronic hepatitis B and C; Follicular lymphoma; Malignant melanoma	Roferon A® Hoffmann–La Roche (Basel, Switzerland)	IFNα-2a (*E. coli*)	1986 (EU) 1986 (USA)
IFNα-2a	Chronic hepatitis B; Chronic myelogenous leukemia; Melanoma	Pegasys® Hoffmann–La Roche (Basel, Switzerland)	PEGylated IFNα-2a (*E. coli*)	2002 (USA and EU)
IFNα-2b	Multiple myeloma; Chronic myelogenous leukemia; Chronic hepatitis B and C; Carcinoid tumor; Hairy cell leukemia; Follicular lymphoma; Malignant	Intron A®, Alfatronol® (Merck Sharp & Dohme Corp., Kenilworth, NJ, USA)	IFNα-2b (*E. coli*)	1986 (USA) 1986 (EU)

Interferon (IFN) Type/Subtype	Clinical Indication	Commercial Name	Active Pharmaceutical Ingredient	Approval Date
	melanoma; Condylomata acuminate; Kaposi's sarcoma			
	Chronic hepatitis B and C	Viraferon® (Schering-Plough Corporation, Brussels, Belgium)	IFNα-2b (*E. coli*)	2000 (EU)
	Chronic hepatitis C	Rebetron® (Schering-Plough Corporation, Brussels, Belgium)	ribavirin/IFNα-2b (*E. coli*)	1999 (USA)
	Chronic hepatitis C	ViraferonPeg® (Merck Sharp & Dohme Corp., Kenilworth, NJ, USA)	PEGylated IFNα-2b (*E. coli*)	2000 (EU)
	Chronic hepatitis C	PegIntron® (Schering-Plough Corporation, Brussels, Belgium)	PEGylated IFNα-2b (*E. coli*)	2001 (USA) 2000 (EU)
	Chronic hepatitis C	Albinterferon®/Albuferon® (Novartis—Basel, Switzerland; Human Genome Sciences, Rockville, MD, USA)	Fusion protein of albumin and IFNα-2b (*E. coli*)	2010 (USA)
	Melanoma	Sylatron™ (Merck & Co., Inc, Kenilworth, NJ, USA)	PEGylated IFNα-2b (*E. coli*)	2011 (USA)

Interferon (IFN) Type/Subtype		Clinical Indication	Commercial Name	Active Pharmaceutical Ingredient	Approval Date
IFNα (I)	IFNα-2c	Chronic viral hepatitis; HIV infection	Berofor® (Boehringer Ingelheim, Lda, Ingelheim am Rhein, Germany)	IFNα-2c (*E. coli*)	1989 (USA)
	IFNα-n3	Condyloma acuminate	Alferon N® AIM ImmunoTech (Philadelphia, PA, USA)	IFNα-n3 *(human leukocytes)*	1987 (USA)
	IFNα-n1 (lymphoblastoid)	Chronic hepatitis B and C; Hairy cell leukemia; HPV infection	Wellferon®Glaxo Wellcome (London, United Kingdom)	IFNα-n1 *(human lymphoblastoid cells)*	1997 (USA)
	IFNα-con-1	Chronic hepatitis C	Infergen® (Three Rivers Pharmaceuticals, Warrendale, USA)	IFNα (*E. coli*) IFNα + Ribavirin (*E. coli*)	2001(USA)
IFNβ (I)	INFβ-1a	Multiple sclerosis	Avonex® (Biogen Idec, Maidenhead, United Kingdom)	IFNβ-1a *(CHO cells)*	1996 (USA) 1997 (EU)
			Rebif® (EMD Serono, London, United Kingdom)	Glycosylated IFNβ-1a *(CHO cells)*	2002 (USA) 1998 (EU)
			Plegridy® (Biogen Idec, Maidenhead, United	PEGylated IFNβ-1a *(CHO)*	2014 (EU and US)

Interferon (IFN) Type/Subtype	Clinical Indication	Commercial Name	Active Pharmaceutical Ingredient	Approval Date
		Kingdom)		
		Betaseron® (Chiron—Emeryville, USA; Berlex Laboratories, Richmond, VA, USA)	IFNβ-1b (differs from human protein® in that Cysteine-17 is replaced by Serine) (*E. coli*)	[3][13][14] 1993 (USA)
	® INFβ-1b®	Multiple sclerosis	Betaferon® (Bayer Pharma, Leverkusen, Germany) ®	1995 (EU) ®
		Extavia® (Novartis Europharm, Camberley, United Kingdom; Novartis Pharmaceuticals, East Hanover, NJ, USA)	IFNβ-1b (*E. coli*)	2008 (US) 2009 (EU)
IFNγ (II)	INFγ-1b	Chronic granulomatous disease; Osteopetrosis	Actimmune® (Vidara Therapeutics, Dublin, Ireland)	1990 (US)
		Imukin® (Boehringer Ingelheim, Lda, Ingelheim am Rhein, Germany)	IFNγ-1b (*E. coli*)	1996 (US)

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ated from infected cells [15], thus assigning the term "*interferon*" to this interfering agent. Later, in 1978, due to improved molecular biology tools and developments on the upstream stage allowed researchers to obtain sufficient amounts of IFN with which to perform a reduced physical and chemical characterization of this biomolecule [16]. IFNs are natural cell-signaling glycoproteins produced by eukaryotic cells in response to viral infections, tumors, and other biological inducers, and thus represent part of the first line of defense of vertebrates against infectious agents [13][17].

IFNs cannot be classified as a single protein [16]; instead, they require use of different letters-α, β, and γ-to refer to the main classes of IFNs, which are, respectively, produced by leukocytes, fibroblasts, and lymphocytes (T cells and natural killer cells) [18]. In 1985, a new class (ω) was introduced in humans [19], and class τ [20] was further

discovered in ovine cells. Furthermore, depending on their properties and their ability to bind to cell receptors, IFNs can also be classified into three different types (I to III), with each type displaying the ability to bind to a specific receptor and to trigger different signal transduction pathways and immunological responses, as shown in Table 2.

Table 2. Classification of interferons based on the type of receptor through which signaling takes place. Adapted from Diamond and collaborators [21].

IFN Type	Class	Discovery Year	Receptor Binding
I	α	1957	High binding affinity to IFNAR2, which then recruits low-affinity IFNAR1 to form the signaling competent ternary complex
	β	1957	
	ω	1985	
	τ	1996	
II	γ	Early 1970s	Affinity for IFNGR (IFNGR1 and IFNGR2)
III	λ1	2003	High binding affinity to IFNLR1, which then recruits low-affinity IL-10Rβ to form signaling competent ternary complex
	λ2		
	λ3		
	λ4		

Briefly, type I IFNs bind to a heterodimeric receptor composed of two chains, IFNAR1 and IFNAR2, leading to the activation of the receptor-associated Janus-activated kinases (JAKs) TYK2 and JAK1, respectively (Figure 1) [22][23][24][25]. The next step in this signal transduction pathway is tyrosine phosphorylation of signal transducers and activators of transcription—STAT1 and STAT2—and the subsequent assembling of the heterotrimeric IFN-stimulated gene factor 3 (ISGF3) transcription factor complex. Distinctly, type II IFNs bind to a different cell-surface

receptor consisting of IFNGR1 and IFNGR2 subunits, which in turn associate with JAK1 and JAK2, respectively, leading to phosphorylation of STAT1 (Figure 1) [26]. Finally, type III IFNs bind to a heterodimeric cytokine receptor composed of an IL-28R-binding chain and IL-10R2 that is shared with the IL-10 family of cytokines (Figure 1) [27]. The signaling cascade is like that of type I IFNs, in which the ISGF3 transcription factor complex binds to ISRE (IFN-stimulated response element) elements in gene promoters to induce transcription of IFN-inducible genes (ISGs). However, coordination and cooperation of multiple distinct signaling cascades, including the mitogen-activated protein kinase p38 cascade and the phosphatidylinositol-3-kinase cascade, are required for the generation of responses to IFNs [13].

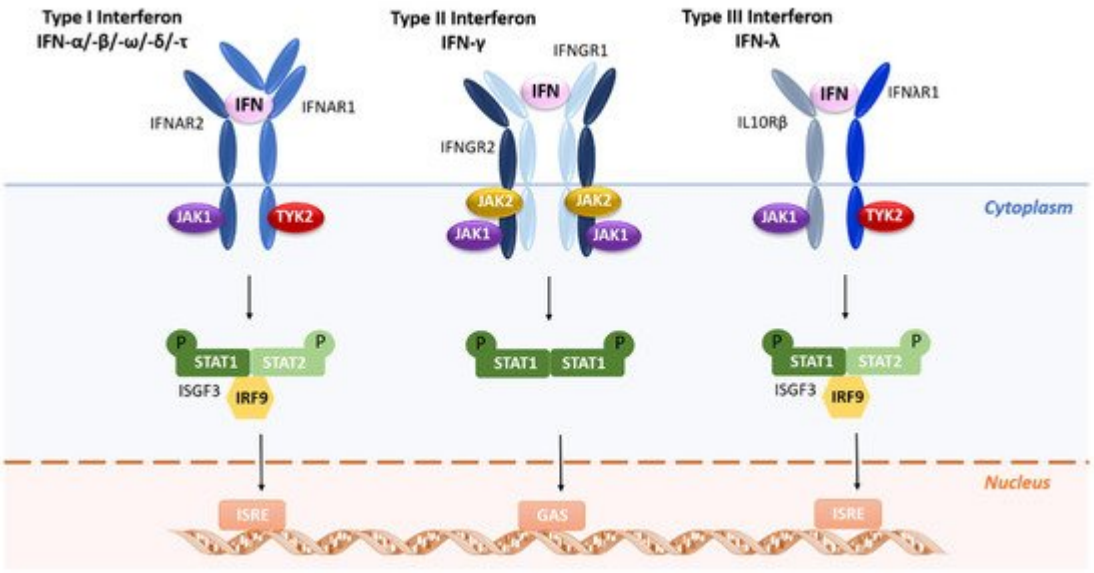

Figure 1. Receptor activation or ligand-receptor complex assembled by type I, type II, or type III interferons.

Since their discovery by Isaacs and Lindenmann, IFNs have been known for their antiviral and antitumoral activities. These proteins own a broad spectrum of activity that impacts cellular metabolism and differentiation, and thus the antitumor effects appear to be due to a combination of direct antiproliferative effects, as well as indirect immune-mediated effects [16][17][28][29]. Accordingly, IFNs have been used in clinical practice to promote immune responses against infections and to treat autoimmune disorders and cancer, among others [16][17]. Furthermore, they can have synergistic or additive effects between them and with other biological response modifiers. The antiproliferative activity of IFN can be classified as direct or indirect [17][29][30], depending on if they inhibit the growth of cancer cells by stopping the cell cycle, apoptosis, or differentiation [17][30], or if they activate immune cells, such as T cells and natural killer (NK) cells, stimulating the immune system against oncogenesis and controlling tumor development [29][30]. The antiviral mechanism of IFN, like the antiproliferative mechanism, is based on the control of gene expression [17]. The antiviral response strongly depends on the virus, the host cell, and the type of IFN. The infection of a cell by a virus induces the production of IFN, which can then exert an autocrine or paracrine action on the surrounding cells. This phenomenon triggers the expression of proteins regulated by this IFN, which collectively constitute, in a very generalized way, the antiviral response responsible for inhibiting virus multiplication [17][28][31]. Schreiber and coworkers [32] determined the binding affinities (to isolated IFN receptor chains 1 and 2)

and biological activity (antiproliferative and antiviral models) of IFNα subtypes. The authors found that the binding affinity and antiproliferative activity correlated with each other, but that for antiviral potency, there were several cases where the relationship appeared to be more complex than simple binding [32]. According to the authors, the concordance of the binding with the activity for most of the subtypes suggests that receptor binding events play a major role in the activity profiles of these molecules [32].

In sum, both the antiviral and antiproliferative mechanisms are based on the regulation of gene expression [28][30]. The proteins produced in response to the transcription and translation of these genes can have a direct or indirect action, leading in the latter case to the joint work of several aspects of the immune system [17][30]. Structural studies [33][34] have shown that type I IFNs consist of five α-helices (labeled A–E), which are linked by one overhand loop (AB loop) and three shorter segments (BC, CD, and DE loops) [23]. The detailed analysis of the structure of this subclass of IFNs revealed similar α-helical cores but large structural differences in AB loops. These insights demonstrate that subtle sequence differences and specific structural rearrangements influence the IFN-receptor interaction and may hold the key for the observed differences in biological activity [23]. Additional details on the structure of IFNs and their influence on IFN biological activities have been reviewed elsewhere [17][23][35][36][37].

3. Therapeutic Cloned Interferons

Commercial IFN-based products were first derived from leukocytes and then from lymphoblastoid lines [36]. However, as both protein extraction from natural producers and chemical synthesis undergoes inherent constraints that limit regular large-scale production, recombinant DNA technologies have rapidly become a choice for therapeutic protein production, including IFNs [38]. The relatively small size (Mw ~20 kDa) and compactness of the IFN protein, combined with the lack of any functional glycosylation (at least in some cases, unglycosylated IFNs are predicted to be functionally identical to their glycosylated counterparts), has contributed to high yield and improved bioactivity [36]. These therapeutic proteins are obtained ex vivo mostly in biological systems and must guarantee, in addition to full protein functionalities, a cost-effective industrial manufacturing in the absence of impurities (host cell proteins, DNA, aggregates, among others) [38].

The complete manufacturing process to obtain recombinant therapeutic proteins comprises four main stages, summarized in Figure 2: (i) the development stage, in which the gene of interest is isolated, cloned in a suitable plasmid, and then the recombinant plasmid is introduced in the selected host, allowing the master cell bank to be obtained; (ii) the production itself, or *upstream* stage, which is associated with the choice of a particular expression system and respective culture conditions; (iii) the *downstream* stage, including the recovery of the target protein, followed by its purification from a heterogeneous and highly complex matrix that generally encompasses chromatographic techniques (corresponding to the most expensive part of the process); and (iv) fill and finish, whereby the final product formulation is developed according to the method of administration, and the process must ensure that the stability and biological activity of the purified biopharmaceutical is maintained during storage and transport [4][39]. Protein drugs must necessarily conform with quality constraints stricter than those expected in the production of enzymes for chemical industries, which consequently defines the choice of recombinant hosts, protocols, and production/purification strategies [38]. Moreover, there is a generic consensus about the need to

enable drugs for cell- or tissue-targeted delivery, aiming for a reduction in dosage, production costs, and side effects [38]. To this end, therapeutic proteins are usually administered in formulations whose compositions are optimized to guarantee improved stability and delivery of target biopharmaceuticals. In general, the purity, activity, and safety of the finished products are ensured by critical aspects, including host cell development, cell culture, cell bank establishment, protein synthesis, purification process, and subsequent protein analysis, formulation, storage, and handling [40].

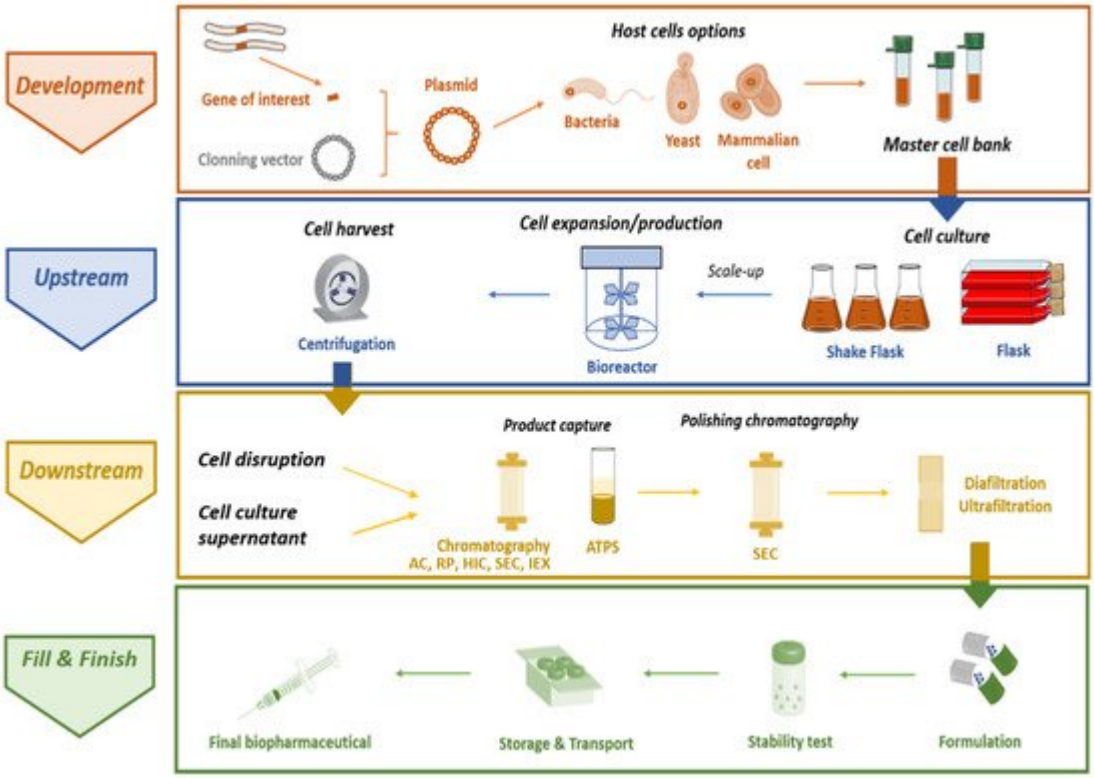

Figure 2. Overview of the manufacturing of IFN-based biopharmaceuticals (ATPS–Aqueous two-phase system; AC–Affinity chromatography; IEX–Ion-exchange chromatography; HIC–Hydrophobic interaction chromatography; RP–Reverse phase chromatography; SEC–Size exclusion chromatography).

References

1. Nathan, C.; Cars, O. Antibiotic resistance--problems, progress, and prospects. N. Engl. J. Med. 2014, 371, 1761–1763.
2. Guiochon, G.; Beaver, L.A. Separation science is the key to successful biopharmaceuticals. J. Chromatogr. A 2011, 1218, 8836–8858.
3. Walsh, G. Biopharmaceutical benchmarks 2018. Nat. Biotechnol. 2018, 36, 1136–1145.
4. Jozala, A.F.; Geraldes, D.C.; Tundisi, L.L.; Feitosa, V.A.; Breyer, C.A.; Cardoso, S.L.; Mazzola, P.G.; Oliveira-Nascimento, L.; Rangel-Yagui, C.O.; Magalhaes, P.O.; et al. Biopharmaceuticals

from microorganisms: From production to purification. Braz. J. Microbiol. 2016, 47 (Suppl. 1), 51–63.

5. Castro, L.S.; Pereira, P.; Passarinha, L.A.; Freire, M.G.; Pedro, A.Q. Enhanced performance of polymer-polymer aqueous two-phase systems using ionic liquids as adjuvants towards the purification of recombinant proteins. Sep. Purif. Technol. 2020, 248, 117051.
6. Buckel, P. Recombinant Protein Drugs (Milestones in Drug Therapy). Molecules 2001, 6, 1063.
7. Lagassé, H.; Alexaki, A.; Simhadri, V.; Katagiri, N.; Jankowski, W.; Sauna, Z.; Kimchi-Sarfaty, C. Recent advances in (therapeutic protein) drug development. F1000Research 2017, 6.
8. Roger, S.D. Biosimilars: Current status and future directions. Expert Opin. Biol. Ther. 2010, 10, 1011–1018.
9. Timmermann, C. How to produce ‘marketable and profitable results for the company‘: From viral interference to Roferon, A. Hist Philos. Life Sci. 2019, 41, 30.
10. Lindenmann, J.; Schleuning, W.-D. Interferon: The Dawn of Recombinant Protein Drugs, 1st ed.; Lindenmann, J., Schleuning, W.D., Eds.; Springer: Berlin/Heidelberg, Germany, 1999; Volume 7, pp. 121–134.
11. Research and Markets. Interferons Global Market Report 2020-30: COVID-19 Implications and Growth. Available online: (accessed on 5 January 2021).
12. ClinicalTrials.gov Database (Search by “Interferon“). Available online: (accessed on 10 January 2021).
13. Ryff, J.-C.; Pestka, S. Interferons and Interleukins. In Pharmaceutical Biotechnology, 5th ed.; Crommelin, D., Sindelar, R., Meibohm, B., Eds.; Springer: Cham, Switzerland, 2019; pp. 619–643.
14. Silva, A.C.; Lobo, J.M.S. Cytokines and Growth Factors. In Current Applications of Pharmaceutical Biotechnology; Silva, A.C., Moreira, J.N., Lobo, J.M.S., Almeida, H., Eds.; Springer: Cham, Switzerland, 2019; pp. 87–113.
15. Isaacs, A.; Lindenmann, J.; Andrewes, C.H. Virus interference. I. The interferon. Proc. R. Soc. Lond. Ser. B Biol. Sci. 1957, 147, 258–267.
16. Pestka, S.; Langer, J.A.; Zoon, K.C.; Samuel, C.E. Interferons and their actions. Annu. Rev. Biochem. 1987, 56, 727–777.
17. Borden, E.C.; Sen, G.C.; Uze, G.; Silverman, R.H.; Ransohoff, R.M.; Foster, G.R.; Stark, G.R. Interferons at age 50: Past, current and future impact on biomedicine. Nat. Rev. Drug Discov. 2007, 6, 975–990.
18. Interferon nomenclature. Nature 1980, 286, 110.

19. Hauptmann, R.; Swetly, P. A novel class of human type I interferons. Nucleic Acids Res. 1985, 13, 4739–4749.
20. Liu, L.; Leaman, D.W.; Bixby, J.A.; Roberts, R.M. A type I ovine interferon with limited similarity to IFN-alpha, IFN-omega and IFN-tau: Gene structure, biological properties and unusual species specificity. Biochim. Biophys. Acta 1996, 1294, 55–62.
21. Lazear, H.M.; Schoggins, J.W.; Diamond, M.S. Shared and Distinct Functions of Type I and Type III Interferons. Immunity 2019, 50, 907–923.
22. Taniguchi, T.; Takaoka, A. A weak signal for strong responses: Interferon-alpha/beta revisited. Nat. Rev. Mol. Cell. Biol. 2001, 2, 378–386.
23. Pestka, S.; Krause, C.D.; Walter, M.R. Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 2004, 202, 8–32.
24. Decker, T.; Müller, M.; Stockinger, S. The Yin and Yang of type I interferon activity in bacterial infection. Nat. Rev. Immunol. 2005, 5, 675–687.
25. Ivashkiv, L.B.; Donlin, L.T. Regulation of type I interferon responses. Nat. Rev. Immunol. 2013, 14, 36–49.
26. Takaoka, A. Cross Talk Between Interferon-gamma and -alpha /beta Signaling Components in Caveolar Membrane Domains. Science 2000, 288, 2357–2360.
27. Uzé, G.; Monneron, D. IL-28 and IL-29: Newcomers to the interferon family. Biochimie 2007, 89, 729–734.
28. Gibbert, K.; Schlaak, J.; Yang, D.; Dittmer, U. IFN-α subtypes: Distinct biological activities in anti-viral therapy. Br. J. Pharm. 2013, 168, 1048–1058.
29. Jonasch, E.; Haluska, F.G. Interferon in oncological practice: Review of interferon biology, clinical applications, and toxicities. Oncologist 2001, 6, 34–55.
30. Bekisz, J.; Baron, S.; Balinsky, C.; Morrow, A.; Zoon, K.C. Antiproliferative Properties of Type I and Type II Interferon. Pharmaceuticals 2010, 3, 994–1015.
31. Samuel, C.E. Reoviruses and the Interferon System. In Reoviruses II: Cytopathogenicity and Pathogenesis, 1st ed.; Tyler, K.L., Oldstone, M.B.A., Eds.; Springer: Berlin/Heidelberg, Germany, 1998; pp. 125–145.
32. Lavoie, T.B.; Kalie, E.; Crisafulli-Cabatu, S.; Abramovich, R.; DiGioia, G.; Moolchan, K.; Pestka, S.; Schreiber, G. Binding and activity of all human alpha interferon subtypes. Cytokine 2011, 56, 282–289.
33. Radhakrishnan, R.; Walter, L.J.; Hruza, A.; Reichert, P.; Trotta, P.P.; Nagabhushan, T.L.; Walter, M.R. Zinc mediated dimer of human interferon-α2b revealed by X-ray crystallography. Structure

1996, 4, 1453–1463.

34. Klaus, W.; Gsell, B.; Labhardt, A.M.; Wipf, B.; Senn, H. The three-dimensional high resolution structure of human interferon α-2a determined by heteronuclear NMR spectroscopy in solution. J. Mol. Biol. 1997, 274, 661–675.
35. Ealick, S.; Cook, W.; Vijay-Kumar, S.; Carson, M.; Nagabhushan, T.; Trotta, P.; Bugg, C. Three-dimensional structure of recombinant human interferon-gamma. Science 1991, 252, 698–702.
36. Platis, D.; Foster, G.R. Interferon Proteins: Structure, Production and Purification. In The Interferons: Characterization and Application; Meager, A., Ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2006; pp. 73–83.
37. Gad, H.H.; Dellgren, C.; Hamming, O.J.; Vends, S.; Paludan, S.R.; Hartmann, R. Interferon-λ Is Functionally an Interferon but Structurally Related to the Interleukin-10 Family. J. Biol. Chem. 2009, 284, 20869–20875.
38. Sanchez-Garcia, L.; Martín, L.; Mangues, R.; Ferrer-Miralles, N.; Vázquez, E.; Villaverde, A. Recombinant pharmaceuticals from microbial cells: A 2015 update. Microb Cell Fact 2016, 15.
39. Rosano, G.n.L.; Ceccarelli, E.A. Recombinant protein expression in Escherichia coli: Advances and challenges. Front. Microbiol. 2014, 5.
40. Owczarek, B.; Gerszberg, A.; Hnatuszko-Konka, K. A Brief Reminder of Systems of Production and Chromatography-Based Recovery of Recombinant Protein Biopharmaceuticals. Biomed. Res. Int. 2019, 2019, 1–13.