The evolution of biologics in the context of oncological therapy

ABSTRACT

Progress in the field of pharmacy, closely related with the mutual stimulation of natural sciences and new technologies available to researchers, has been so rapid over the last few decades that it has begun to cause problems at the level of definitions and classifications. This phenomenon refers also to the term of biologics or, more widely, biopharmaceuticals (in Polish terminology). The first associations with the above terms lead our thoughts to recombinant proteins, such as insulin used in the treatment of diabetes or monoclonal antibodies with wide, in terms of therapeutic areas, applications. It is generally believed that the above category of drugs is not associated with preparations invented long before the discovery of nucleic acids, let alone before the invention of an ordinary bulb. Importantly, the connotation of the term biopharmaceuticals is undergoing a very rapid reconstruction before our eyes, and the set of referents is expanding with newer, previously unknown types of therapies. Technological progress is one of the driving forces of these changes. Unmet medical needs, including the ones in the area of oncology, constitute another driving force.

Key words: biologics, biopharmaceuticals, recombinant proteins, monoclonal antibodies, CAR-T, gene therapy

A brief history of biopharmaceuticals

In the broadest sense, the history of biopharmaceuticals, although uncaptured and unclassified as part of meta-science for many years, begins as early as the second half of the 18th century. Its beginning is synonymous with the bold achievements of Edward Jenner, an English physician, who believed in underestimated folk wisdom, according to which the history of cowpox (a contagious viral disease of domestic cattle and pigs) gave immunity to smallpox. Thus, Jenner used material from people infected with animal smallpox to develop the first effective vaccine against deadly smallpox [1]. As a matter of fact, there are reports indicating that the first vaccination was made by a farmer named Jesty, 22 years before Jenner himself. However, it was Jenner who is, due to his striving for the spread of his discovery and his approach based on a scientific method, widely recognised as the inventor and precursor of the application of products of biological origin [2].

Another great breakthrough occurred in the 1940s when the development of technology was driven by the world-engaging war. The needs of the front and the necessity to gain an advantage on it involved huge investments in research based on the observations of Alexander Fleming. Although he discovered bactericidal mould as early as in 1928, the interest in his work first by Ernst Chain and Howard Florey, and later also by Norman Heatley, an English biochemist, came in the late 1930s [3]. After presenting the research results of the team of these scientists at the University of Oxford, American pharmaceutical companies became interested in penicillin. However, after internal evaluation, none of these companies continued studying the issue. It is only the interest from the American War Production Board that changed the course of history. Contracts for mutual exchange of information between Merck, Squibb, Pfizer, Midwest, Abbott Laboratories, Upjohn, Parke, and Davis were signed. The method of production in milk cans was replaced by a large-scale
manufacturing process based on a highly effective fungal strain selected during the development. By the end of 1944, penicillin demonstrated its usefulness in military use, and in March 1945 it entered the domestic and foreign markets [4].

The beginning of the rapid development of biological therapies is, however, associated with a completely different therapeutic area than infectious diseases. It refers to the application of insulin in the therapy of diabetes. Scientific intuition led researchers to discover insulin from the second half of the 19th century, when Paul Langerhans characterised a group of pancreatic cells of distinct structure as compared to the remaining ones. This group was later named the islets of Langerhans, in honour of its discoverer, by Gustaw Laguesse, a French pathologist [5]. Edward Albert Sharpey-Schäfe, an English physiologist, observed afterwards that they produced a substance capable of lowering blood sugar levels. Consequently, in line with the emerging terminology, he used the name applied in 1909 by Jean de Meyer and introduced the term of insulin to medicine (Latin: Insula — island). The newly discovered molecule began to gain medical and commercial significance only as a result of the work of scientists from Toronto: Frederick Banting, Charles Best, and James Collip. They developed a method of insulin extraction from the pancreas of animals based on optimised alcohol concentration. Even before clinical trials were completed, Eli Lilly’s production facilities started to manufacture the protein, and then to introduce the innovative therapy to the pharmaceutical market in 1922 and revolutionise the approach to diabetes treatment. In the subsequent decades, it was possible to sequence and synthesise insulin, which, combined with the discoveries attributed to Watson and Crick, caused another revolution at the end of the 1970s. In 1978, the company Genentech, originating in California, a pioneer in the field of pharmaceutical biotechnology, in cooperation with the City of Hope National Medical Centre, developed the first insulin using recombinant DNA technology. Thanks to this, as early as in 1982, the above-mentioned Eli Lilly, as a licensee, implemented the first recombinant drug called Humulin, produced in a bacterial expression system, opening a new era in the development of pharmacy [6].

**The era of DNA recombination**

Almost immediately after insulin, recombinant human growth hormone (Protropin; 1985) and interferon-alpha variants (Roferon A, Intron A; 1986) were introduced. Production of recombinant vaccines was started as well (Recombivax; 1985). The 1980s and 1990s were the times of the so-called first-generation biopharmaceuticals — recombinant proteins identical in structure to native human proteins, mainly hormones, cytokines, enzymes, growth factors, and blood coagulation factors. In the second half of the 1990s, excluding several previous cases, the so-called second-generation biopharmaceuticals entered the market, i.e. molecules with a modified sequence, exchanged sugar residues, surface modified molecules through a covalent bond with polyethylene glycol, and so-called fusion proteins, being the combination of two or more sequences. The objective of the above variations was to improve efficiency, reduce the number of side effects, and achieve better pharmacokinetic parameters. Exemplary molecules are fast-acting (Humalog; 1996) and long-acting (Lantus; 2000) types of insulin, and pegylated interferons alpha (Peg-Intron; 2000 and Pegasys; 2002). The instances of even more technologically complex solutions include etanercept, i.e. the fusion of the crystallisable fragment (Fc) of the IgG1 antibody (immunoglobulin) with fragments of the tumour necrosis factor (TNF) receptor (Enbrel; 1998) and the fusion of diphertheria toxin with interleukin-2 (IL-2) (Ontak; 1999) [7].

Initially, prokaryotic expression systems based mainly on *E. coli* strains were used to produce biopharmaceuticals based on recombinant DNA technologies. They enabled, in a relatively inexpensive way, the acquisition of so-called high-density cell cultures, from which, after disintegration of bacterial cells, most often using chromatographic techniques, the target therapeutic proteins were purified. Although the *E. coli* system has been successfully applied to produce numerous molecules to this day, over the years, with the increase in complexity of the drug structure (molecular weight, post-translational modifications, complex fusions) and attempts to eliminate the problem of immunogenicity, more demanding methods in the form of the eukaryotic expression systems have started to be used. These were strains of *S. cerevisiae* (e.g. lutropin — Luveris), BHK cell lines, i.e. Baby Hamster Kidney (e.g. blood coagulation factor VIII — Kogenate), and, above all, CHO cell lines, i.e. Chinese Hamster Ovary, which is of utmost significance for development of antibody production methods (few examples of mAbs molecules are based on the hybridoma system).

Thus, progress in molecular biology and biotechnology generated over 200 biologics by 2015. Their sales reached an incredible value of 196 billion USD in 2015, which accounted for 29% of the market for all drugs. This value exceeded the estimates of market analysts — in the report ‘Global Protein Therapeutics Market Forecast to 2015’ published in 2012 by RNCOS, it was estimated that the biopharmaceuticals market would reach 143 billion USD in 2015 [8]. *Kelly Scientific Research* estimates from 2015 point to further increases
— the value of the biological drug market is expected to reach 463 billion USD in 2021, accounting for 32% of the entire drug market (Fig. 1) [9]. Importantly, at the end of 2015, over 900 new biopharmaceuticals in the form of protein molecules and antibodies, cellular therapies, as well as gene and antisense therapies were under development. Over 5000 subsequent projects were subject to early laboratory evaluations at that time. It cannot be disputed that the discussed field is still developing very rapidly. However, biologics have already found their application in many therapeutic areas and individual indications, including the following: diabetes, neutropaenia, thrombocytopaenia, anaemia, hepatitis, growth deficiency, myocardial infarction and heart failure, strokes, and a number of autoimmune diseases. Their application in cancer treatment is also growing (Fig. 2).

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**Definition of biopharmaceuticals as well as benefits and problems associated with their use**

According to the FDA, biopharmaceuticals (biologics, biopharmaceuticals, biological medical products) are products generated by and isolated from living organisms. They can be of natural origin (human, animal, or microorganism) or produced with the application of biotechnological methods. They include the following: vaccines, blood components, tissues, cells, gene therapies, and therapeutic proteins (including antibodies). They can have a structure based on proteins and peptides, sugars, nucleic acids, or their complexes or combinations. They can also be living structures, such as tissues and cells. Due to the development of the technology of producing recombinant molecules over the past 30 years, the definition has been reduced
to therapeutic recombinant proteins and monoclonal antibodies. From the point of view of both the system and the physician, as well as the patient, these solutions are not free from defects. The complicated manufacturing process affects both the cost of drug development and the treatment itself. According to data provided by Kelly Scientific Research, in 2015 the average cost of the treatment of a patient with biologics was 20–55 times higher than the cost of treatment based on so-called conventional therapy (small chemical molecules) [9]. In addition, biopharmaceuticals, due to lower stability than conventional medicines, usually require compliance with more stringent storage procedures and preparation for administration to the patient. Hospitalisation and observation are more frequently required. However, their market success is not unfounded. In many cases, as a rule, when the molecular target of the drug is a structure that does not require penetration through the cell membrane (most often the surface receptor or its soluble ligand), biologics are the best tool to achieve this goal by eliminating the non-specific interactions that can cause a whole range of adverse effects. These are, by definition, targeted drugs. What is more, due to their natural structure, despite a longer, usually favourable half-life period associated with molecular weight, they are degraded and eliminated from the body without the risk of accumulation and long-term deposition in the body’s tissues. Also, they do not penetrate the normal blood-brain barrier. However, the latest scientific knowledge and technology that allows the manipulation of sugar residues in the production process, enable the development of the molecules burdened with the problem of immunogenicity to a significantly lesser extent.

Cytokines and immunotoxins

The first biopharmaceuticals used in oncology were the above-mentioned recombinant variants of alpha interferons. Roferon A and Intron A have been applied in the treatment of specific leukaemias, lymphomas, sarcomas, melanomas, and kidney tumours. By the end of the 1990s, interferons and erythropoietins constituted the largest share of the recombinant drug market. However, over the years, as existing therapies were improved and new therapies were introduced, particularly in well-developed countries, it was not possible to maintain this dominance. As interferons are a group of proteins from the cytokine family involved in numerous processes related to the activation of elements of the immune system, their application is associated with an average number of side effects that are very burdensome for the patient. Thus, despite high dynamics of the entire market and the fact that the market value of interferons alone increased (from 5.7 billion USD in 2002 to 8.6 billion USD in 2009), their total share in the market of biologics is systematically falling. In 2002, it was over 17% [11], in 2009 only 7% [8], and in 2015 less than 5% [10]. These calculations should also take into account the fact that they apply to all interferons (including beta and gamma) and the fact that more than half of the sales of alpha interferons is associated with treatment of viral infections (mainly hepatitis and AIDS). In terms of these indications, subsequent generations of alpha interferons, such as Peg-Intron and Pegasys (pegylated forms of alpha interferons) were developed.

Another example of cytokines in oncological therapy, developed in the 1990s and approved for the first time in 1992, is interleukin-2. A molecule called aldesleukin has found application in the treatment of metastatic kidney cancer and melanoma [12]. Recombinant IL-2 also became part of the structure of a product called Ontak (Denileukin Diftitox) constituting the recombinant fusion of a cytokine with the diphtheria toxin-related domain approved by the FDA in 1999 in the treatment of primary cutaneous lymphomas [13]. The same product was withdrawn by the FDA in 2014.

In the context of attempts to implement the concept as closely as possible to the ideal of targeted therapy, on the wave of achievements in the area of so-called small molecules and development of Gleevec, subsequent research projects were less frequently directed towards non-specific immunotherapy. From the perspective of today’s researchers, this approach was relatively brutal. The subsequent programs required both completely different molecular goals and ways of their accomplishment.

Cluster of differentiation

Rituximab was the first representative of the new direction. As part of the mechanism of action of this molecule, the idea of targeting is implemented by using a cluster of differentiation antigens and hits the CD20 present on B lymphocytes. Hence, next to autoimmune diseases, the huge potential of rituximab is noticed in the treatment of non-Hodgkin’s lymphoma and lymphocytic leukaemia. What is very important is that structurally this molecule is a monoclonal antibody capable of inducing antibody-dependent cell cytotoxicity (ADCC), due to the presence of the crystallisable fragment (Fc domain) [14]. According to IgeaHub estimates for 2018, the total annual sales value of rituximab (Rituxan and Mabther) was to be about 8.1 billion USD. In 2017, under the name of Biogen and Genentech, Rituxan Hycela with recombinant hyaluronidase enabling rapid subcutaneous administration, reached the market.

At the same time as the first rituximab was under development, cluster of differentiation met with inter-
est from other groups active in the drug discovery field. CD52 was applied as a molecular target for the drug Campath (alemtuzumab) used to treat B-CLL (B-cell chronic lymphocytic leukaemia). It was launched on the market in 2001 [15]. The next examples, however, represent an even higher level of structural engineering. Catumaxomab (Removab), in addition to the CD3 antigen present on T-lymphocytes, binds the EpCAM (epithelial cell adhesion molecule) protein — a molecular target present on the cell surface of many types of neoplasms. Catumaxomab is a trifunctional antibody for which each of the antigen binding fragments (Fab) have an affinity for a different molecular target [16]. Blinatumomab (Blincyto) constitutes an even more unusual construction. This molecule is a representative of Bi-specific T-cell engagers (BiTE) antibodies and consists of two single-chain variable fragments (scFv) of antibodies linked by a peptide linker. This molecule does not have a crystallisable fragment (Fc) of the antibody. One of the variable fragments is responsible for binding of the CD19 antigen that is subject to expression on the surface of B lymphocytes in acute lymphoblastic leukaemia, and the other for recruitment of T-lymphocytes by direct interaction with the CD3 antigen [17]. An interesting example of attempts to increase the potential of antibodies targeting cell differentiation antigens is the product Adcetris (brentuximab vedotin) manufactured by Seattle Genetics. This drug belongs to the group of ADCs (antibody drug conjugates). It is a monoclonal antibody directed against CD30 (an antigen present on Hodgkin lymphoma cells, cutaneous T-cell lymphoma, and anaplastic lymphoma) conjugated by maleimide with monomethylated auristatin E [18].

**Growth factors**

Almost in parallel with the concept of molecules targeting clusters of differentiation, attention was paid to the possibility of using monoclonal antibody technology against a completely different group of molecular targets, elements of the growth factor signalling pathways available outside the cell receptors or their ligands. As signal transmitters, these pathways constitute an important stimulus in the emergence and progression of numerous neoplastic diseases. The flagship example of a drug developed in accordance with this concept is Herceptin (trastuzumab) used for 20 years for breast cancer with expression of the gene encoding HER2 (human epidermal growth factor receptor 2). It is a murine humanised antibody directed against HER2 — one of the receptors of the EGFR family (epidermal growth factor receptor). In 2013, a bioconjugate that was structurally based on Herceptin, in which the crystallisable fragment (Fc) of the same antibody was combined with thiols with a small molecule inhibitor of the mitotic cell division, mertansine, was launched on the market. The new ADC is available under the trade name Kadcyla. The activity of the next molecule, which can be used in combination with trastuzumab in breast cancer, is directed to the same HER2 receptor, but to a different epitope. Pertuzumab (Perjeta), first introduced in 2013, blocks a fragment of the HER2 receptor responsible for interaction and dimerisation with HER3, therefore preventing the formation of the most active form of the complex capable of transmitting the pro-survival signal [19]. Other instances of exploration of the EGF family of ligand pathways are cetuximab (Erbitux) and panitumumab (Vectibix). Both molecules are anti-EGFR monoclonal antibodies. The first is a chimeric molecule; the other one is fully human. They are applied in metastatic colorectal cancer with overproduction of EGFR and KRAS wild type.

An example of implementation of the slightly different strategy for monoclonal antibodies is bevacizumab (Avastin) developed by a team from Napoleon Ferrara. For many years, it was one of the blockbusters among drugs in general. In addition to broad indications in oncology, it is applicable in ophthalmology, in age-related macular degeneration (AMD). Unlike the previously indicated examples, Avastin, according to the postulated mechanism of action, does not target growth factor receptors, but rather their ligands — specifically, vascular endothelial growth factor A (VEGF-A). It was marketed in 2004 as the first angiogenesis inhibitor. It was approved in the treatment of rectal and colorectal cancer, non-small cell lung cancer (NSCLC), kidney cancer, glioblastoma multiforme, and breast cancer. It was withdrawn from the last indication by the FDA in 2010 [20]. Another example of the molecule targeting the VEGF pathway is ramucirumab (Cyramza), a fully human anti-KDR (kinase insert domain receptor) antibody. This quite new angiogenesis inhibitor was approved for the treatment of some gastrointestinal cancers and NSCLC in 2014.

Significantly, two angiogenesis inhibitors applied in oncology have registered indications for treatment of age-related macular degeneration (wet AMD). Bevacizumab and Ziv-Aflibercept (Zaltrap) are present on the market in this way. The second one on the ophthalmic drug market is known as Eylea. It is a fusion protein consisting of the IgG1 Fc domain combined with two soluble receptor fragments. It is a VEGF-Trap type construction, additionally capable of interacting with PGF (placental growth factor). In oncology, Zaltrap is applied to treat metastatic colorectal cancer [21].

**Immune checkpoints**

A completely new, ground-breaking, and currently intensively explored strategy in oncology is the applica-
tion of immune checkpoint inhibitors for therapy. It is thanks to them that the neoplasm, in the development process, creates an immunosuppressive environment around itself in which the immune system becomes inactive towards it. Therefore, blocking the checkpoints by turning off receptors or ligands that negatively regulate immune cell function should, by definition, make the neoplasm visible and vulnerable again [22].

Ipilimumab (Yervoy), which constitutes an antibody directed against CTLA-4 (CD152; cytotoxic T-lymphocyte-associated protein 4), a protein present on the surface of T-lymphocytes, which have been activated by contact with an antigen, is the first recombinant molecule approved in 2011, striking a completely new type of molecular target. Ipilimumab, by blocking CTLA-4, prevents the lymphocytes from transmitting a negative feedback signal by APC (antigen presenting cells), due to which neoplastic cells are not recognised as their own. Thus, lymphocyte deactivation does not occur. Ipilimumab is approved in the treatment of inoperable melanoma and kidney cancer [23].

PD-1 (programmed cell death protein 1) signal inhibitors function on the basis of a simpler mechanism. Nivolumab (Opdivo), pembrolizumab (Keytruda) and cemiplimab (Libtayo) are directed at the PD-1 receptor, present on the surface of the activated lymphocytes. This receptor is responsible for negative regulation of the immune response. Inactivation of the receptor by antibodies prevents recognition of the neoplastic cells as their own, by blocking the interaction with the PD-L1 ligand present on them [24]. By the time cemiplimab was approved in 2018, the other two molecules had already broadened their indications and were intensively conquering the market. As estimated by Evaluate, Keytruda, and Opdivo, sales are expected to reach 9.17 billion USD and 7.8 billion USD, respectively, in 2019 (Fig. 3).

The most intuitive approach in the group of checkpoint inhibitors, based on targeting the neoplasm itself, and not directly the cells of the immune system of the patient, is represented by atezolizumab (Tecentriq), durvalumab (Imfinzi), and avelumab (Bavencio). These particles are targeted at PD-L1. They prevent its interaction with PD-1 and CD80.

Thus, in just a few years, the market for immune checkpoint inhibitors filled with as many as seven molecules. Subsequent players are forced to make difficult business decisions related to the selection of indications. It should be noted that PD-1 and CTLA-4 are not the only molecular targets under this approach to treatment of neoplasms. May the next molecules based on LAG-3, TIM-3, B7-H3/4, and BTLA signalling constitute another breakthrough.

Table 1 shows selected representatives of various classes of oncological biologics. Figure 4 presents oncological biologics with the highest sales value in 2017.

**Figure 3.** Biologics among the 10 drugs with the highest forecast sales value in 2019 (based on [25])

**CAR-T and the future of oncological treatment**

In 2017, the FDA issued a approval in B lymphoblastic leukaemia derived from B lymphocytes for the first Novartis (Kymriah) therapy based on CAR-T technology. As part of the treatment, the patient’s T-lymphocytes are collected and genetically modified so that additional receptors (Chimeric antigen receptors) appear on their surface, in this particular case directed at CD19. Afterwards, the cells return to the patient. In the Evaluate’s 2018 report on the list of the most promising research programs, two further CAR-T projects in the Celgene pipeline in the third phase of clinical development are mentioned: bb2121 (anti-BCMA) and JCAR017 (anti-CD19). There are many more similar programs in preclinical development, and the interest in CAR technology is growing [25].

We are certainly at a very interesting point in the history of oncological biologics. The achievements of
Table 1. Selected representatives of specific classes of biologics for cancer treatment in chronological order. Source: Authors' own compilation

| First approval | Molecule | Trade name | Structure | Molecular target | Company               |
|----------------|----------|------------|-----------|------------------|----------------------|
| 1986           | IFN alfa 2a | Roferon A  | Rh-interferon alfa 2a | IFN-alfa receptor | Roche                |
| 1986           | IFN alfa 2b | Intron A   | Rh-interferon alfa 2b | IFN-alfa receptor | MSD                  |
| 1992           | Aldesleukin | Proleukin  | rIL-2      | IL-2 receptor     | Chiron/Novartis      |
| 1994           | Filgrastim  | Neupogen   | rhG-CSF    | G-CSF receptor    | Roche                |
| 1997           | Rituximab   | Rituxan    | mAb        | CD20             | Roche                |
| 1998           | Trastuzumab | Herceptin  | mAb        | HER2             | Roche                |
| 1998           | Thyreotropin alfa | Thyroogen | rhTSH alfa | TSH receptor | Genzyme              |
| 1999           | Denileukin difitox | Ontak | rIL-2-diiptheria toxin | IL-2 receptor, EF-2 | Eisai               |
| 2001           | Alemtuzumab | Campath    | mAb        | CD52             | Bayer                |
| 2002           | Peg-filgrastim | Neulasta | PEG-rhG-CSF | G-CSF receptor   | Amgen                |
| 2004           | Bevacizumab | Avastin    | mAb        | VEGF-A           | Roche                |
| 2004           | Cetuximab   | Erbitux    | mAb        | EGFR             | Merck                |
| 2006           | Panitumumab | Vectibix   | mAb        | EGFR             | Amgen                |
| 2009           | Catumaxomab | Removab    | mAb        | CD3, EpCAM       | Fresenius            |
| 2011           | Ipilimumab  | Yervoy     | mAb        | CTLA-4           | Bristol-Myers Squibb|
| 2011           | Brentuximab vedotin | Adcetris | ADC         | CD30             | Seattle Genetics     |
| 2012           | Ziv-afibercept | Zaltrap | Fc(IgG1)-VEGF-Trap | VEGF-A, VEGF-B, PGF | Sanofi               |
| 2013           | Pertuzumab  | Perjeta    | mAb        | HER2             | Roche                |
| 2013           | Trastuzumab emtansine | Kadcyla | ADC         | HER2             | Roche                |
| 2014           | Nivolumab   | Opdivo     | mAb        | PD-1             | Bristol-Myers Squibb|
| 2014           | Pembrolizumab | Keytruda  | mAb        | PD-1             | MSD                  |
| 2014           | Ramucirumab | Cyramza    | mAb        | VEGFR2           | Lilly                |
| 2014           | Blinatumomab | Blincyto  | BiTE       | CD19, CD3        | Amgen                |
| 2016           | Atezolizumab | Tecentriq | mAb        | PD-L1            | Roche                |
| 2017           | Durvalumab  | Iminfinzi  | mAb        | PD-L1            | AstraZeneca          |
| 2017           | Rituximab hyaluronidase | Rituxan Hycela | mAb + rh-hyaluronidase | CD20 | Biogen/Genentech |
| 2017           | Avelumab    | Bavencio   | mAb        | PD-L1            | Pfizer/Merck         |
| 2018           | Cemiplimab  | Libtayo    | mAb        | PD-1             | Regeneron/Sanofi      |

Figure 4. Biologics in oncology with the highest sales value in 2017 (based on [26])
scientist in recent years have given birth to new drug delivery technologies and have pointed to completely new, previously underestimated or unknown molecular targets. Improvement as part of the development of the so-called ‘biobetters’ will also apply to already tested drugs. From the point of view of the cost of therapy and availability for the patient, approvals of biosimilar drugs should be important. Unfortunately, in oncology, only three molecules have appeared on the European market: trastuzumab (Ontruzant) from Samsung Bioepis, rituximab (Rixathon) from Sandoz, and rituximab (Truxima) from Celltrion. Thus, the market is still within a very narrow group of manufacturers who care about their interests.

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