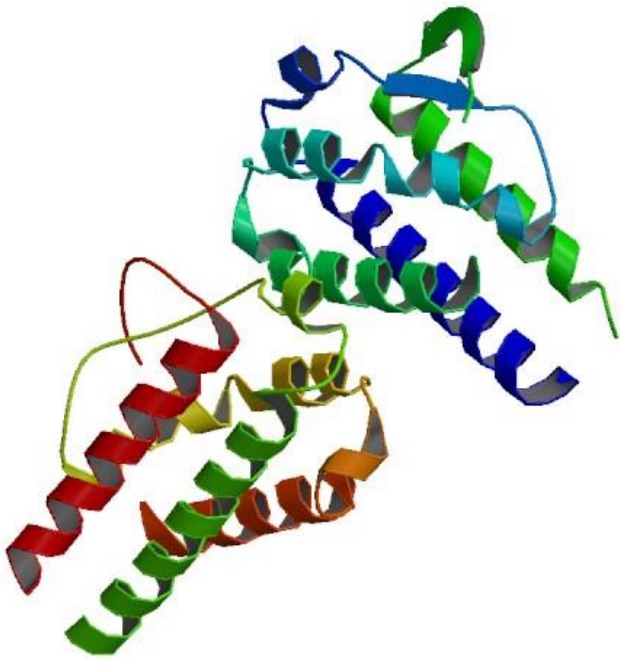

INTERLEUKIN-2

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Biology term Paper
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1. Preface

1.1 What is our motivation to work on the topic we chose?

Interleukin 2 (IL-2) has several potential uses in cancer therapy. This in itself presents an interesting topic since the fight to end cancer is of great importance in today's society. Furthermore cancer touches a personal spot in many people's lives because it is so common.

1.2 What is especially interesting?

Interleukin-2 is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". These characteristics are of great relevance in IL-2's effectiveness against cancer. Aldesleukin, a recombinant of IL-2, has been approved by the U.S. Food and Drug Administration (FDA) and in several European countries for the treatment of cancers (malignant melanoma, renal cell cancer) in large intermittent doses and has been extensively used in continuous doses. IL-2 has a narrow therapeutic window, and the dosage usually determines the severity of the side effects. Severe and dangerous side effects like breathing problems, serious infections or seizures have been noted when IL-2 is used in higher doses.

1.3 What are our questions in respect to the chosen topic?

- Will Interleukin-2 prevail as an efficient tool used in cancer treatment?

- Is the method ready for large scale usage?

2. Introduction

2.1 What is the context?

IL-2, also known as T-cell growth factor (TCGF), is a cytokine, the first ever interleukin to be discovered and classified. IL-2 is part of the body's natural response to microbial infection and in differentiating foreign cells from the body's own cells. Interleukin-2 is a protein, regulating the activities of white blood cells responsible for the body's immune activity. Permitted in Austria, Germany and Switzerland, Aldesleukin is a DNA recombinant produced drug for the treatment of renal cell and metastatic melanoma. Proleukin is the trading name, operated and created by Novartis AG (DocCheck Flexikon, 2016). Many other DNA recombinant drugs based on IL-2 are currently in research such as Darleukin (Pilogen, 2016).

2.2 Where and why is it used?

Aldesleukin is used for the treatment of renal cell and metastatic melanoma. There are three main areas where it is effective:

- interfering the growth and multiplication of the cancer cells
- improving the growth of killer T cells and other cells which attack the cancer cells
- manipulating cancer cells to send out specific chemicals which attract the immune system cells

(Cancer Research UK, 2016; CenterWatch, 2016)

2.3 Alternative treatments

Commonly used cancer treatments are:

- **chemotherapy**
- **surgery**
- **radiation therapy**
- **targeted therapy**
- **immunotherapy**

Other procedures and techniques include

- **stem cell transplant**
- **hyperthermia**
- **photodynamic therapy**
- **blood product donation and transfusion**
- **lasers in cancer treatment**

(American Cancer Society, 2016)

3. Description of engineering technique on the example of Aldesleukin

In order to produce Aldesleukin, a human interleukin-2 coding DNA strand (plasmid) is isolated and placed in a genetically modified E. coli cell (Fig.1).

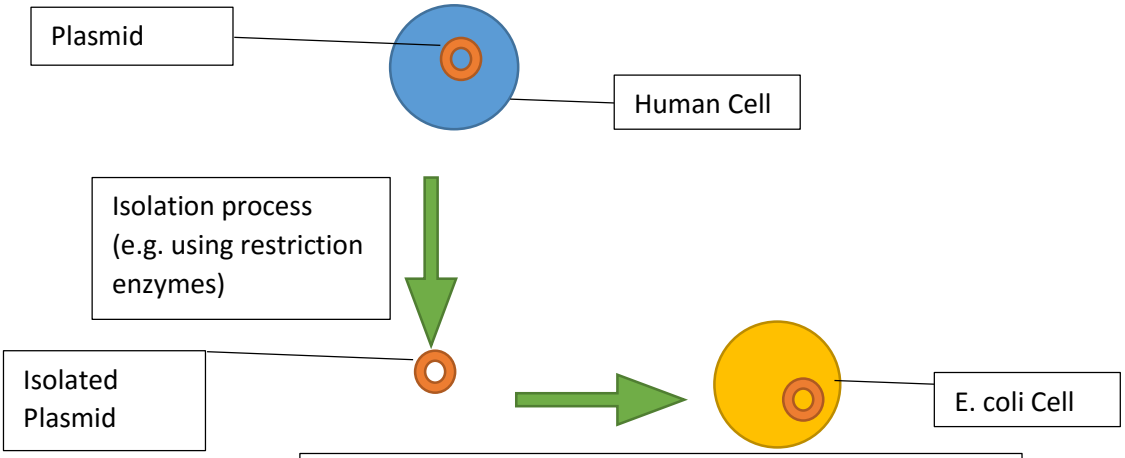

Fig.1: First step in the production of Aldesleukin

In the E. coli cell two main changes will occur to the plasmid (Fig.2).

1. The sequence coding for cysteine at position 125 will be substituted by serine.
2. The codon for the N-terminal alanine will be cut out.

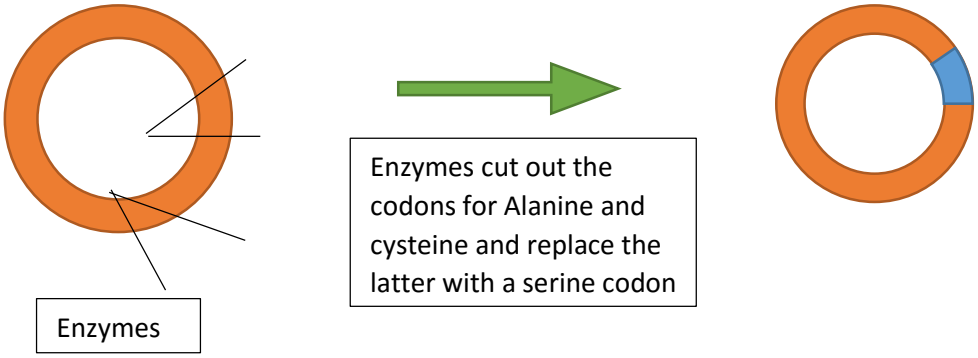

Fig. 2: Second step in the production of Aldesleukin: DNA recombination

The bacteria will now start with the protein synthesis, 2 changes may be occurring:

1. It will not glycosylate the protein it is synthesizing.
2. It may change its aggregation state.

Finally a recombinant version of interleukin-2, Aldesleukin, is left.

(Prometheus Laboratories Inc., 2015; DrugBank, 2016)

4. Documentation and pictures of the research institution

4.1 Interview with Katharina Frey

1. **How has the research for Interleukin-2 as drug (Aldesleukin) advanced in the last few years? Have there been any recent discoveries?**
   „After I left ETH, my colleague Katrin Gutbrodt continued with the F8-IL2 project and focused on the targeting of AML (leukaemia) which for the first time also includes not only solid tumours. After performing experiments in mice in combination therapy with cytarabin or cemadotin (Gutbrodt 2013, 2014), a different antibody-IL2 version has been tested in clinical trials in patients with AML. In her study, immunocompetent mice (=mice with normal immune system including T, B, NK cells) showed complete eradication of tumour. (In immunocompromised/immunodeficient mice you will get only tumour killing based on NK cells, and not of T cells since they are not present in these mice. You need immunocompromised mice for growing human tumours which would be rejected by mice with complete immune system. The latter ones are used for growing of mouse tumours).
   Since F16-IL2 (and not F8-IL2) has already been tested and has all certificates to be tested in humans, F16-IL2 was used to treat patients with AML (Schliemann paper and Gutbrodt 2013). 1 out of 4 patients had no tumour after 3 months, 2 of 4 a tumour shrinkage and one of 4 two months tumour-free before relapse. These data also showed a strong local presence and activation of immune cells which is caused by the IL2-part of the antibody-IL2 fusion protein.
   Despite AML, IL2-based antibodies have been tested in other types of cancer in patients http://www.philogen.com/en/products/pipeline/pipeline_16.html, e.g. as direct injection (together with L19-TNF) in melanoma (Pretto 2014) and head & neck cancer patients, or as systemic therapy in melanoma (with dacarbazine) but also in breast and lung cancer. I attached a review from Thomas List with an overview of immunocytokines in clinical trials.
   Antibody-IL2 is always superior to IL2 treatment alone for the fact that it is targeted and in addition it is larger in size and therefore not so rapidly excreted via urine and therefore, circulates longer in the body."

2. **Will the research continue to work on Interleukin-2 to look for other uses of it?**
   „Yes for sure, IL2 is a working horse at Philochem and the most potent cytokine for T and NK cell activation, the most potent killer cells in the body."

3. **Is the manufacturing process of Aldesleukin from Interleukin-2 a costly and complicated one, since it is so expensive to buy? What is your opinion regarding this?**
   „Every time when you have to produce biologics (in cells or bacteria), you need very specialized nutrition medium and growth circumstances and control conditions, plus extensive purification steps from the medium and activity testing's in order to get a fully active and always reproducible batch of the drug. This is very cost intense, much more than a fully chemical drug such as Aspirin for example. Furthermore, also all

clinical developments and trials which cost millions and billions of Euros have to be covered with the costs during the patented period (in total 20 years after patenting, usually the company has 10 years where a drug is commercialized and when they earn money.) That is the reason why new drugs always cost a lot as compared to old ones."

4. **Do you think that IL-2 will become better accessible in the future? Why/Why not?**
   „With respect to the format, I think IL2 itself will at some point be used less due to strong side effects (fever, headache, etc.) in the patients and will be overcome by better tolerated or especially targeted forms of IL2, having the best therapeutic response, not as single agent alone but always together with another drug in combination therapy. It is always a matter of money vs. treatment strategy vs severity of disease.
   As far as cancer in general is concerned, you will not be able to cure cancer just with one drug, you always have to tackle and attack at different points: tumour cells, immune system, blood system, different signalling pathways, and tumour cell DNA damage.
   What do you mean with accessible? As long as the health insurance pays in the developed countries, I hope that every needed therapy will be covered and payed. In countries without health insurance, all expensive treatments will only be affordable for the rich people. And in countries with poor infrastructure, you also have to take simple things into account such as the availability of fridges and freezes, enough medical doctors, and good diagnostic medical centres."

5. **IL-2 was once thought to work against HIV, but the work on it was dropped, saying that it would be ineffective. Do you think that this is true or was it just a lack of work and/or funding?**
   „The HIV virus is very intelligent. It always hides in CD4 T cells and develops strategies to overcome the immune system control and HIV therapies. IL2 might only work on top of another drug, but for my feeling, it is too less efficient and too less potent. So I think it is true, but don't forget about side effects for the patients and that HIV patients have to use drugs daily. So, would you like to always have fever chills and headaches? It is also always about improvement of quality of life (also in cancer therapy, and it starts already when you can formulate a drug as tablet which you can take at home and not as an injectable drug for which you have to go to the hospital)."

4.2 Photographs of the lab

Since we were not able to have a live interview, Ms Frey kindly sent us some of her pictures she had taken at the time of her PhD

The lab in which Katharina Frey and her team worked on Interleukin-2.

Katharina Frey during her work.

5. Discussion

5.1 Progress made with the application

Progress has been made in the sense that the usefulness of Aldesleukin has been approved by various research conducting institutions, which has motivated companies in the pharma industry to start further research in the field of IL-2 recombinant drugs. For example Philochem is currently experimenting with Darleukin, a potential IL-2 recombinant drug. Aldesleukin today is widely used as a biological therapy drug against kidney cancer. Since it has already presented itself as effective against kidney cancer, it is now also in clinical trials for some other types of cancer. We can safely say that advances have been made in this branch of medicinal science, but the research is still in its early phase and the race for breakthrough discoveries has only just begun.

5.2 Future Steps

Research continues in the field of IL-2. It is a working horse at Philochem and the most potent cytokine for T and NK cell activation, the most potent killer cells in the body. After the success of Aldesleukin, efforts now concentrate on various other recombinants of IL-2. The graph in Fig 3 illustrates the present stages of various products. As soon as research hits PHASE III the product gets approved, enrolled and eventually brought to the market.

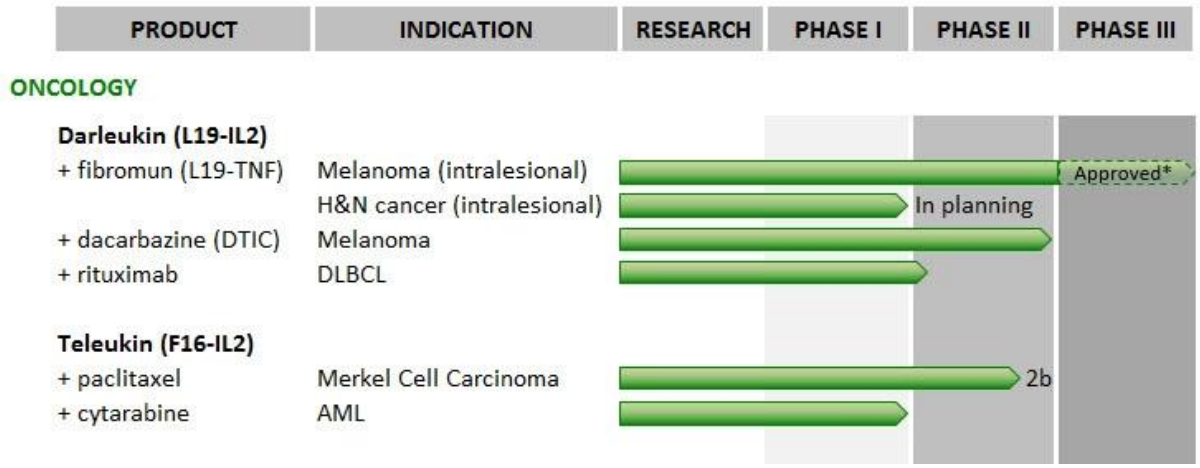

Fig. 3: Diagram showing the advancement of different IL-2 recombinants (Philogen, 2016)

5.3 Ethical aspects

Advantages	Disadvantages
• Effective cure for some types of cancer • Very potent • Research will go on for a fair amount of time	• Very high production costs →high prices→unaffordable • Side effects • Animal experiments (like every other medication)

6. Summary

Since the discovery of Interleukin 2 (also called T cell growth factor, TCGF) in 1976, interest in this peptide hormone produced by the body's T-cells has continually increased. IL-2 and especially its recombinant drug Aldesleukin (first FDA approved in 1992) have shown effectiveness in certain cancer therapies and are promising further potential uses in the medical field.
IL-2's most important trait for cancer therapy is its ability to differentiate between foreign ("non-self") and "self" within the body.
Proleukin is the commercial name for the drug which is produced by Novartis AG. Numerous other DNA recombinant drugs based on IL-2 are in research right now.
On the cellular level, Aldesleukin causes a series of events that then stimulate growth and differentiation of T-cells.
Today Aldesleukin is commonly used as a biological therapy drug in treating kidney cancer and metastatic melanoma and is currently in clinical trials for other types of cancer. Present research, mostly motivated by the success of Aldesleukin, concentrates on finding new recombinants of IL-2. In regards to ethical aspects IL-2 and it's recombinant drugs aren't very controversial, but there are still some pro and contra arguments which can be made: their potency and big potential for future discoveries as clear advantages, but also their high production costs and side effects as definite drawbacks.
Ongoing research in the field of immunology and t-cell biology will most likely be followed by the development and approval of new drugs and promising advances in the field of cancer therapy.

7. References

- American Cancer Society (2016). Treatments and side effects. http://www.cancer.org/treatment/treatmentsandsideeffects/index [21/04/2016]
- Cancer Research UK (2016). Aldesleukin (IL-2, Proleukin or interleukin 2). http://www.cancerresearchuk.org/about-cancer/cancers-in-general/treatment/cancer-drugs/aldesleukin-or-il2 [15/04/2016]
- CenterWatch (2016). Proleukin. http://www.centerwatch.com/drug-information/fda-approved-drugs/drug/391/proleukin [15/04/2016]
- DocCheck Flexikon (2016). Interleukin-2 http://flexikon.doccheck.com/de/Interleukin-2 [15/04/2016]
- DrugBank (2016). Aldesleukin**.** http://www.drugbank.ca/drugs/DB00041 [15/04/2016]
- Herati, Ramin (2006). IL2 Crystal Structure. https://en.wikipedia.org/wiki/File:IL2_Crystal_Structure.png [15/04/2016]
- Philogen (2016). PIPELINE. http://www.philogen.com/en/products/pipeline/pipeline_16.html [15/04/2016]
- Prometheus Laboratories Inc. (2015). PROLEUKIN ® (aldesleukin). http://www.proleukin.com/assets/proleukin.pdf [15/04/2016]