NeutroCure Newsletter 2020

Welcome to our first Newsletter, celebrating the progress of NeutroCure! Our goal is to develop improved drugs that for an effective immune system. An over-active immune system may lead to chronic inflammation and the cytokine storm phenomenon that can be observed during infection. Our hypothesis is that resolution of inflammation is dependent on the timely production of reactive oxygen species (ROS) by the first inflammatory cell on the scene, the neutrophil, to maximise clearance of pro-inflammatory debris.

On January 1st 2020, the partners in our Future Emerging Technology programme, NeutroCure, led by Erlangen and from were looking forward to an eventful year of exciting collaborative science.

At our kick-off meeting in Erlangen, Germany, on January 31st 2020, we met face to face. We carefully reviewed our commitments, early deliverables and resolved outstanding administrative matters before receiving in depth presentations from each of the partners detailing their commitments. Members attending the meeting were (1) Andriy Mokhir; (2) Malin Hultqvist; (3) Helen Griffiths; (4) Markus Hoffmann and Martin Herrmann; (5) Rostyslav Bilyy; (6) Andrés Hidalgo Alonso.

We know that if ROS production is insufficient, for example when the enzyme NOX2 is absent or mutant, then inflammation persists. Central to our project is the development of new technology to resolve inflammation - novel ROS-amplifiers that are designed as single- and multiple-trigger-dependent prodrugs targeting different organelles within cells. Two classes are being designed, synthesised, formulated and evaluated during the project. The first class of these acts independently of NOX2 and is based on N-alkylaminoferrocene (NAAF).

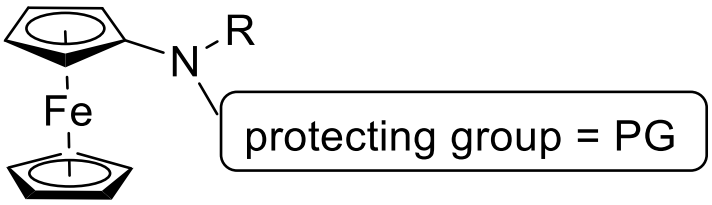

Figure 1. NeutoCure Prodrug – NOX-2 independent

The second NOX2-dependent class includes molecules targeting formyl peptide receptors e.g. FPR1

Progress in synthesis

Much of the activity planned for the first year has centred on design, synthesis and simple screening of novel compounds and progress has been good.

The partners had not envisioned the disruption due to the covid-19 pandemic, and the different impact on each of the partners. The need for additional face protection and social distancing when laboratory work was an important aspect of ensuring safety for individuals working in the teams (Figure 2).

Three new co-workers were employed to work on the synthetic aspects of the project in Erlangen.

Mr. Bohdan Golub, a PhD candidate (MSc-degree in chemistry from Kyiv Shevchenko University, Ukraine),

Mr. Anton Arkhypov, a PhD candidate (MSc-degree in chemistry from Friedrich-Alexander-University of Erlangen-Nuremberg, Germany),

Ms. Marlies Ripple, a MSc candidate (BSc-degree in chemistry from Friedrich-Alexander-University of Erlangen-Nuremberg, Germany).

Figure 2. Professor Mokhir's team in Erlangen, adopting covid-secure practices

Despite the COVID restrictions, the first prototypes of unspecific NOX2–independent ROS amplifiers were prepared and tested in cell free setting, in cells and in a preclinical model of inflammation in vivo by Erlangen, enabling the successful delivery of deliverables D2.2 and D2.3 by the end of June 2020.

Since ROS are highly reactive and their production is not easily measurable in vivo, we have explored the potential to use a class of lipid oxidation biomarkers, the oxysterols, that can be either generated by ROS e.g. 7-keto cholesterol or by enzymes during inflammation e.g. 25 hydroxycholesterol. Methods have been established that enable us to determine oxysterol production in different subcellular organelles (references 2 and 3). Using this approach, we will be able to explore the localization of ROS production in neutrophils.

Also, and according to the plan, a medium sized library (~50 compounds) of ROS amplifiers activated in the presence of several neutrophil-specific triggers was generated and first tests in cell free settings were conducted, which confirm their expected functionality by December 2020.

The first prototypes of unspecific NOX2-activating ROS amplifiers were also anticipated, and the success of and FPR1 activator was reported in the Journal of Leukocyte Biology (reference 1 below).

Progress in models

In addition to well-established pre-clinical models of inflammation that are used extensively by different labs, we aimed to develop a further in vivo model to explore the efficacy of killing of cancerous cells mediated by neutrophils and effect of our novel compounds. This work is led by Lviv National University and the model is summarized in Figure 3 below.

Figure 4 describes the novel tumor model to study neutrophil-mediated drug action. A few models of Nemeth-Kellner Lymphoma-based tumors were established successfully in ascites (lymphoma) and also as solid tumor (sarcoma or air pouch). They showed different times of development and will enable different treatment possibilities to be explored (Fig 4A). If grown in a solid type the tumor is highly vascularized and is highly infiltrated with neutrophils (Fig 4B and C).

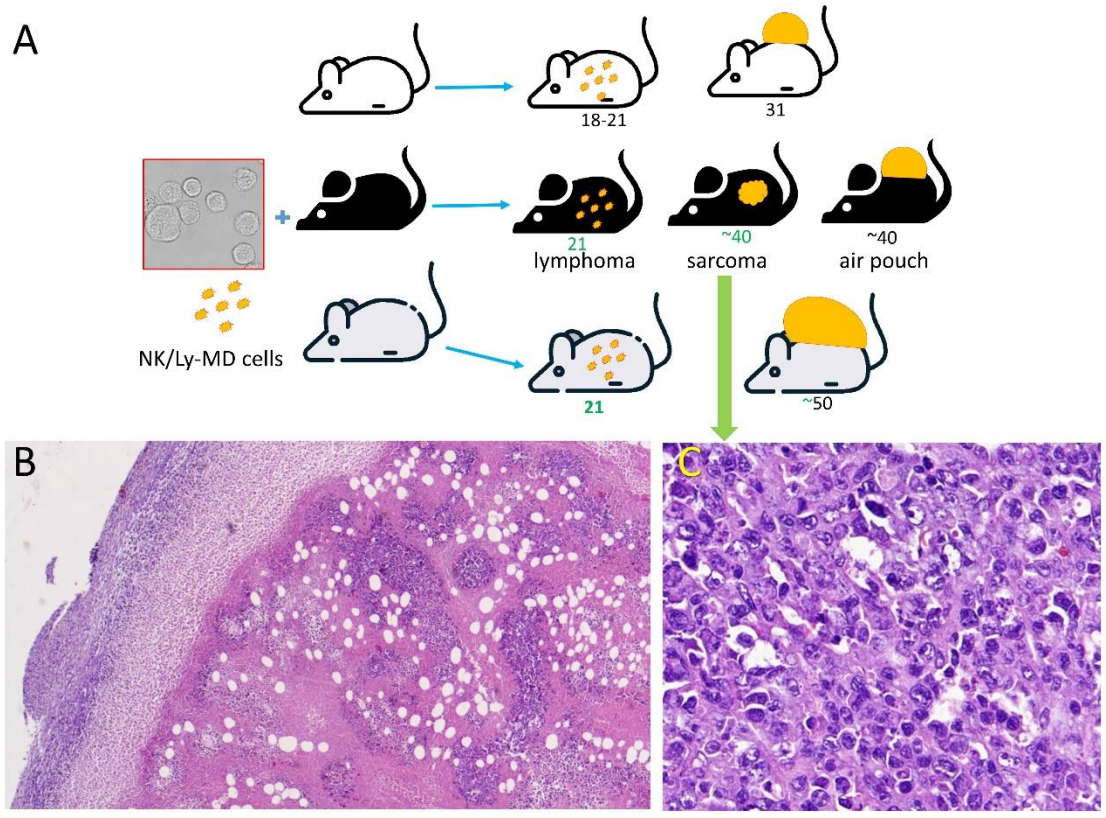

Figure 4. Nemeth-Kellner Lymphoma-based tumors

Further studies from the Lviv group during the first year of the project are reported in references 4 and 5.

Consortium Meetings

On July 17th2020, we held an additional meeting to review progress with deliverables and accountability. The partners agreed to amendments that are now pending the final approvements by the EU.

On December 4th 2020, we held our first successful annual symposium by zoom. It was an opportunity for early career researchers and new members of the teams across Europe to join and gain a wider understanding of the project and its progress.

The zoom format worked well and provided a good framework for extensive discussion.

Agenda - NeutroCure Symposium 1

- 09:10 – 09:15 Opening remarks/ Housekeeping: Helen Griffiths
- 09:15 – 09:45 Organizational topics – Project Deliveries to date/discussion. Andriy Mokhir
- 10:00 Presentation 1 (Andriy Mokhir)
- 10:20- 10:40– Q & A/Discussion
- 10:40 Presentation 2 (Malin Hultqvist)
- 11.00 – 11:20 - Q & A/ Discussion
- 11:20 - 12:00– Presentation 3 (Markus Hoffmann & Martin Herrmann)
- Q & A/ Discussion
- **12:00 Lunch**
- 13:40- 14:00 – Presentation 4 (Helen Griffiths)
- 14:00 – 14:20 – Q & A/ Discussion
- 14:20 – 14:40 Presentation 5 (Rostyslav Bilyy)
- 14:40 – 15:00 - Q & A /Discussion
- **15:00 Break for refreshments**
- 15:15 - Presentation 6 (Andrés Hidalgo Alonso)
- 15:35- 15:55 - Q & A/Discussion

Administration

The group of Mokhir has been responsible for coordination/management/administration of the project including:

- preparation of grant and consortium agreement,

- project initiation,

- budget distribution,

- preparation of the first amendement,

- organization of a series of meetings.

Publications arising

1. Lind S, Dahlgren C, Holmdahl R, Olofsson P, Forsman H. Functional selective FPR1 signaling in favor of an activation of the neutrophil superoxide generating NOX2 complex. J Leukoc Biol. 2020 Oct 11. doi: 10.1002/JLB.2HI0520-317R. Online ahead of print.

2. Borah K, Rickman OJ, Voutsina N, Baple EL, Dias IH, Crosby AH, Griffiths HR. Datasets of whole cell and mitochondrial oxysterols derived from THP-1, SH-SY5Y and human peripheral blood mononuclear cells using targeted metabolomics. Data Brief. 2020 Oct 16;33:106382. doi: 10.1016/j.dib.2020.106382. eCollection 2020 Dec. PMID: 33134439 **Free PMC article.**

3. Borah K, Rickman OJ, Voutsina N, Ampong I, Gao D, Baple EL, Dias IH, Crosby AH, Griffiths HR. A quantitative LC-MS/MS method for analysis of mitochondrial -specific oxysterol metabolism. Redox Biol. 2020 Sep;36:101595. doi: 10.1016/j.redox.2020.101595. Epub 2020 Jun 1. PMID: 32574926 **Free PMC article.**

4. Bilyy R, Bila G, Vishchur O, Vovk V, Herrmann M. Neutrophils as Main Players of Immune Response Towards Nondegradable Nanoparticles. Nanomaterials (Basel). 2020 Jun 29;10(7):1273. doi: 10.3390/nano10071273. PMID: 32610567 **Free PMC article.** Review.