19-20 May, 2021

Symposium Presentation

Abstracts

This symposium was held under the leadership of Ege University Faculty of Medicine, Department of Medical Biochemistry. Department of Histology and Embryology, Research and Education Laboratory (AREL), Cellular Treatment and Regenerative Medicine Commission, Ege University Cord Blood-Tissue Application and Research Center and the Institute of Health Sciences- Department Stem Cell contributed to the arrangement

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Symposium Program

19 May, 2021

13:00 Opening Ceremony

- **Prof. Necdet Budak** – Rector of Ege University (TURKEY) (Honorary President of the Symposium)
- **Prof. Dr. Cemil Gürgün** - Dean of Ege University Faculty of Medicine (TURKEY)

13:30 Prof. Gülinnaz Ercan (Symposium President) " Cellular Therapies and Cancer"

14:00 Assist. Prof. Arnika Kathleen Wagner "Genetic Modification of Natural Killer Cells for Immunotherapy"

15:00 Prof. Evren Alıcı "Strategies to optimize Natural Killer Cell based therapies in cancers"

16:00 Didem Çakırsoy "Preclinical Data of NK-92 and HASSASIN (CD16-IL12) CAR Models Against Solid Tumors"

16.30 Dr. Koray Yalçın "Clinical Application of NK-92 and HASSASIN (CD16-IL12) CAR Models Against Solid Tumors"

17.00 Closing ceremony

20 May, 2021

09:00 Opening Ceremony

09:30 Assoc. Prof. Bülent Özpolat "Development of Targeted -Therapies For Solid Tumors."

10:30 Prof. Engin Ulukaya "The fate of the cell that cannot die: Cancer"

11:30 Prof. Güneş Esendağlı "Immunological adaptation of cancer cells to anti-tumor immunity"

12:30-13:30 Break Time

13:30 Prof. Gamze Tanrıöver "Private life of cancer cells: we love spying... "

14:30 Prof. Nihal Karakaş "New Era for Therapeutic Delivery: Bioengineered Stem Cells"

15:30 Dr. Meng-Wei Ko "Triumph ver Cancer natural killers cells"

16.00 Dr. Meng-Wei Ko "Preferential selection and expansion of $CD8^+$ T cells by osteoclasts-induced"

16:30 Dr. Ege Çubuk "Effects of natural killer cells differentiated from cord blood hematopoietic stem cells on glioblastoma tumor cells, An *in vitro* study."

17.00 Closing ceremony

Cellular Therapies and Cancer

Gulinnaz ERCAN*

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The field of cellular therapy is evolving rapidly with novel therapeutic modalities. Therapies based on the use of autologous immune cells are among the best candidates for cancer therapy including tumor-infiltrating lymphocytes (TILs), engineered T-cell receptor (TCR), chimeric antigen receptor (CAR)-T cells, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Among these, CAR T-cell therapy was the first to be approved by the US Food and Drug Administration (FDA) for the treatment of patients with B lymphoid malignancies. However, a number of challenges have hindered their widespread use in malignancies. Some of these challenges include the lack of ideal targets for solid tumors, antigen loss mediating cancer relapse, the complexity of generating a patient-specific cell product, toxicity owing to the "on-target off-tumor" reactivity and cytokine release syndrome/neurotoxicity, inefficient trafficking to tumor sites, and tumor-mediated immunosuppression. Advances in synthetic biology and genetic engineering, and the investigation of new platforms for cellular therapies leads to the development of strategies to bypass some of these challenges, in order to advance the field forward and make a wider clinical impact. Recent advances in different forms of cellular therapies employing both T and NK cells against hematologic malignancies as well as solid tumors are encouraging. The emergence of synthetic biology approaches for cellular engineering provides a broadly expanded set of tools for programming immune cells for enhanced function. Advances in T or NK cell engineering, genetic editing, the selection of optimal cell source, stem cells and cell manufacturing have the potential to broaden cell–based therapies for cancer.

CAR-T and CAR-NK cell therapies are on the verge of becoming powerful immunotherapeutic tools for combating hematological diseases. Currently, more than 500 CAR-T and 17 CAR-NK cell trials are being conducted worldwide. Most CAR-T cell-based gene therapy products that are under clinical evaluation consist of autologous enriched T cells, whereas CAR-NK cell-based approaches can be generated from allogeneic donors and from stem cells as well. Besides modification based on a second-generation CAR, more advanced CAR-immune cell therapeutics are being tested, not leading to graft-versus-host disease (GvHD) and avoiding therapy resistance caused by antigen loss. These achievements are encouriging for improving cellular immunotherapies for cancer treatment.

Genetic Modifications of NK cells for Immunotherapy

Arnika K. Wagner*

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Immunotherapy is treatment that uses the body's own defense to combat cancer. Although treatments of this type were attempted more than a century ago, its only recently that immunotherapy has shown promise to curing cancer and autoimmune diseases.

There has been a large number of approved immunotherapy classes introduced over the last couple of years, and a lot of them have been targeted towards T cells and have given us unprecedented evidence that one can harness the immune system to have impact in immunotherapy. By bringing in components of the innate immune system, one may really boost current and future immunotherapy approaches. This could be accessory cells of the innate immune system to trigger and increase T cell responses, but also alternative killer cells such as Natural Killer (NK) cells. A historical perspective of NK cell discoveries from a Karolinska Institute perspective has been provided. Basic NK cell biology is discussed, in particular inhibitory and activating receptors and the importance of CD16 for antibody dependent cellular cytotoxicity (ADCC). NK cell interactions with the tumor and cells of the tumor environment are elucidated, with an emphasis on NK cell functions shaping the adaptive immune response to cancer.

For improvement of endogenous NK cell functions, genetic modifications are a promising tool. In order to improve transduction efficiencies of lentiviral vector transductions, temporary inhibition of the RIG-I pathway was shown to be effective. With the use of small molecules of RIG-I inhibition, NK cell transductions can be greatly improved. We are currently developing and testing a second-generation RIG-I pathway inhibitor, which improves genetic modification significantly. This enables CRISPR Cas9 screening in NK cell lines and primary expanded NK cells. In addition, introduction of an artificial receptor, such as chimeric antigen receptor (CAR), is simplified. We have produced CD38-CAR-NK92 and CD38-CAR primary NK cells using a clinically approved retroviral vector. CD38-CAR NK cells show increased activity against CD38+ target cells, and are a promising tool in the fight against CD38+ multiple myeloma. We are currently evaluating the use of these CD38-CAR-NK cells as potential.

Strategies to optimize Natural Killer Cell based therapies in cancers

Evren Alıcı*

*Department of Medicine, Huddinge, Karolinska Institutet, SWEDEN

New immunotherapies against cancer, currently dominated by CAR-T and so-called "checkpoint inhibition" (monoclonal antibodies to inhibitory molecules on immune cells), are in some cases very effective and even curative. However, these therapies are fraught with challenges, both in terms of cost and side effects challenges. At Karolinska Institutet in Huddinge, our group has brought together leading translational and clinical researchers with a strong background in NK cell related research. Our goal is to (i) develop, (ii) produce, and (iii) clinically implement the next generation NK cell-based immunotherapies for the treatment of cancer, with a primary focus on treatment-refractory hematological (myeloid and lymphoid) malignancies. The goal is to develop clinical therapies with high efficiency, lower production costs than existing CAR-T cell therapies, and at the same time lower side effects. The research is based on recently conducting "first-in-human" NK cell-based clinical trials at Karolinska Institutet / Karolinska University Hospital in Huddinge. Current projects have the potential to generate results that are even better than current clinical trials with "first generation" NK cell-based immunotherapies, with the potential to eventually treat selected treatment-refractory hematological malignancies.

Preclinical Data of NK-92 and HASSASIN (CD16-IL12) CAR Models Against Solid Tumors

Didem Çakırsoy*

Acıbadem Labcell Cellular Therapy Center

The clinically applicable NK-92 cell line may provide a valuable alternative to primary NK cells since they can easily be expanded to high numbers and maintained for therapeutic use while retaining consistent phenotypic and functional features. In preclinical studies, NK-92 cells exhibited persistent anti-tumor activity against different hematologic malignancies and some cancers of solid tumor origins. In addition, the safety of infusion of irradiated NK-92 cells was demonstrated in early phase clinical trials. Patented product HASSASIN cell line was designed, manufactured, and used for clinical trials by Acıbadem Labcell. NK-92 cells were transfected with IL-12 and CD16 gene constructs and this new cell line was named Hassasin. Hassasin cells an interesting option for CAR engineering which provides the cells with antigen-specific targeting, thus further enhancing their anti-tumor activity. In preclinical studies, it has been shown that irradiated GD2 CAR Hasassin has a higher tumor-killing effect compared to GD2 CAR NK-92 cells. Moreover, when GD2 CAR Hassasin in the presence of PMBC encounters a tumor cell, with the help of the IL12 expression the ratio of activated CD4 and active CD8 in the environment has been shown to increase. When GD2 CAR Haşşaşin encounters tumor cells, it has been demonstrated that the cytokine response in the environment is a TH-1 response.

As a new perspective for solid tumor cellular therapy, GD2 CAR macrophage has the potential to initiate the cascade of immune reaction after phagocyte or bite the tumor cell. Expression of CARs in human macrophages could redirect their phagocytic function and result in a targeted anti-tumor therapeutic effect with the potential to stimulate an adaptive immune response. Studies have shown that PBMC-derived M1 GD2 CAR macrophage has an increased tumor-killing capacity. In vitro studies of CAR macrophages have proven promising for the treatment of solid tumors and it encouraged further studies in this area.

Clinical Application of NK-92 and HASSASIN (CD16-IL12) CAR Models Against Solid Tumors

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Cellular therapy is the new source of hope in cancer treatment. In the last two decades there are several trials for testing the efficacy and safety of cellular therapies in different tumor models. Natural killer (NK) cell is one of the pioneers in this field. In currrent practice, NK cells are isolated from peripheral or cord blood and also alternatively, NK cell lines could be used as "off-the shelf therapy". There are different types of NK cell lines and NK-92 is the most known and tested one. We prefer NK-92 cell line in our practice because it is reachable, easy to manipulate, and proven to be safe in several clinical trials. Here we present our clinical data with pure and genetically modified NK-92 models. Our results show that NK-92 applications are safe without any severe adverse events. Although solid tumors seem to be more susceptible than hematological malignancies, high tumor burden may play a restrictive role in response. Combination therapies with chemotherapy or monoclonal antibodies could be a subject of upcoming trials. Although systemic administration of NK-92 cells has low efficacy, intratumoral injections in selected cases show promising results for further applications.

Development of Targeted -Therapies for Solid Tumors

Bülent Özpolat*

*MD Anderson Cancer Center, Department of Experimental Therapeutics, USA

Cancer is one of the top two causes of deaths in the US and the world. Cancer therapy includes standard therapies such as surgery, chemotherapy and targeted therapies, by antibody and small molecule inhibitors, and immunotherapy (i.e., check point inhibitors, CAR-T cell therapy). More recently RNA-based targeted therapeutics were approved by FDA. Although there are about more than 100 targeted therapies, due to significant heterogeneity in patient tumors even in the same subtype of cancers, only faction of patient can benefit from targeted therapies due to lack of target expression in all patients. Therefore, gene targeting RNA therapies such as microRNA and siRNA may overcome difficulties seen by the use of small molecule and antibodies provide promising new avenue for targeting these oncogenes. After several decades of research finally some of the RNA targeted therapies emerged and are being tested in clinical trials. Over the last decade our studies have focused on two major oncogenomic kinases, such as KRAS and Elongation Factor-2 kinase (EF2K) that we identified as a major oncogenic driver and molecular target and validated it as a therapeutic target in various solid tumors including breast, pancreatic, lung, and ovarian cancers. The talk will also give background in targeted therapies used in cancer patients and development of novel targeted therapies using noncoding RNAs (siRNA and microRNA) and small molecule-based targeted therapies on the several highly aggressive cancers including triple negative breast cancer, pancreatic, and lung cancer. I will also mention that these therapies sensitize tumor cells to NK-based therapies.

The fate of the cell that cannot die: Cancer

Engin Ulukaya*

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One of the hallmarks of cancer is the resistance to apoptosis. Apoptosis is a natural cell death process that takes place in the organism if a cell is completed its life span. However, cells may escape apoptosis through many mechanisms (e.g. expression of antiapoptotic proteins, expression of decoy receptors, expression of antiapoptotic members of Bcl-2 family or down regulation of proapoptototoic members of Bcl-2 family etc). Cell death is an important process in both biological and medical science although it has been ignored for many years. The monitorization of it has a great potential to open up new horizons. The investigations into the relationship between different types (apoptosis, necrosis, postmitotic cell death, autophagy) of cell death and the diseases is in increase recently. Among the diseases, the place of cell death in cancer treatment is especially promising. For example, an apoptosis marker called caspase-cleaved cytokeratin 18 (also named as M30 or ccK18) has been reported to release into the serum following cell death/apoptosis resulted from the action of anticancer drugs. Therefore, it has been claimed that serum M30 could be used as a pharmacodynamic biomarker of apoptosis[1]. Taking this into account, it may be a wise idea to use it as a tool for the prediction of response to chemotherapeutics, even the survival, in lung or breast cancer patients[2,3]. However, M30 on its own may not be good enough to provide a reliable data in all the cases because of the fact that some tumors may respond to the same treatment in a different way, for example necrosis, rather than apoptosis, or even autophagic cell death. In the case of necrosis, different cell death marker should be employed. M65 ELISA assay has been introduced for his aim. M65 represents to the intact cytokeratin 18 that is released into the serum from the necrotic cells. However, there may still be some obstacles to fully understand the amplitude of cell death following chemotherapy because the level of neither M30 nor M65 increases although the patients respond to treatment very well[4]. In this talk, the reason for this will be discussed.

1. Linder S, Olofsson MH, Herrmann R, Ulukaya E. Utilization of cytokeratin-based biomarkers for pharmacodynamic studies. Expert Rev Mol Diagn. 10: 353-359, 2010

*2. **Ulukaya E**, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival.* Lung Cancer. *56:399-404, 2007.*

*3. **Ulukaya E,** Karaagac E, Ari F, Oral AY, Adim SB, Tokullugil AH, Evrensel T. Chemotherapy Increases Caspase-Cleaved Cytokeratin 18 in the Serum of Breast Cancer Patients.* Radiology and Oncology *45: 116-122, 2011*

*4. Cevatemre B, **Ulukaya E**, Sarimahmut M, Oral YA, Frame FM. The M30 Assay does not detect apoptosis in epithelial-derived cancer cells expressing low levels of cytokeratin 18. (Accepted by Tumour Biology)*

Private life of cancer cells: we love spying...

Gamze Tanrıöver*

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Tumor cells have special properties that normal cells do not have. The most remarkable of these are tumor cells has resistance to cell death, unlimited replicative potential, ability to invasion and migration. These abilities provide suitable conditions for tumor cells to grow and metastasize. Many studies on tumor cells try to understand how these abilities are used of these cells.

We perform lots of different assay to understand the change in cell viability in *in vitro* studies. Cell viability tests performed by reduced tetrazolium salts to formazan such as MTT, MTS and WST-1. In MTT and MTS tests, the reduction of tetrazolium occurs with intracellular mechanism. However, in the WST1 experiment, the reduction of tetrazolium occurs with extracellular mechanism. Tumor cells are known to evading from apoptosis. The two most commonly used mechanisms to investigate the apoptosis of tumor cells are Annexin V-PI labeling and measuring caspase 3/7 activity. In the Annexin V-PI labeling, not only apoptotic cells but also the stage at which cells are in the cell cycle can be identified.

It is important to know the angiogenesis mechanisms for tumor cells to reach nutrients and oxygen. In the recent years, to understand the angiogenesis in the tumor microenvironment, techniques based on mathematical calculations have been tried. For these formulations, tumor density, vascular density in the tumor microenvironment and interstitial fluid pressure are calculated and compared with *in vivo* models. Tumor cells also have invasion and migration potential. While mostly the wound healing assay is used for migration; for invasion, special inserts covered with matrigel are used.

In our studies, we injected to metastatic breast cancer cells to mice orthotopically. Tumor growth can be detected and tumor weights are measured. Some of the markers about proliferation, apoptosis and metastasis expressions were evaluated immunohistochemistry in primary tumor and metastatic organs. Also, microscopic lung and liver metastasis were also evaluated.

In conclusion, starting a cancer study, the first thing is to choose the right methods to prove this hypothesis. Understanding the *in vitro* journey of tumor cells sheds light on guiding *in vivo* models.

New Era for Therapeutic Delivery: Bioengineered Stem Cells

Nihal Karakaş*

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The standard care of cancer involve surgical resection (for solid tumors) and adjuvant chemo- and radiotherapy. These traditional treatments often result in several side effects and high tumor recurrence rates. Alternative therapeutic approaches have been developed and one of the effective way is the targeting therapies against cancers. This strategy can provide selective inducing of cancer cells to death, angiogenesis, recrucuitment of immune cells and/or blocking tumor cell metastasis. One of the promosing cytotoxic fusion proteins is targeted toxins which can be constructed by replacing the receptor binding domain with a cancer selective ligand, an antibody or an antibody fragment. Beside encouraging pre-clinical outcomes, Phase III clinal trials with targeted toxins were failed due to off target delivery, systemic toxicity and half life of the molecules. Afterwards we and others showed that cell based delivery systems provide an option to secrete therapeutics continously and can be directed towards tumor foci specifically. In this context, we developed Pseudomonas exotoxin fused cytotoxins and examined their efficacy in a panel of cancer cell lines expressing the target receptor. Our results showed that PE cytotoxins can function on any cell line with the cognate receptor. Furthermore, we engineered toxin resistant cells by using CRISPR/Cas9 gene editing tool and showed that the toxin resistant cells can express and secrete functional toxins. Taken together, these results potentiate that cell based delivery of PE cytotoxins can be harnessed for several cancers.

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Triumph Over Cancer: Natural Killer Cells

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Cancer is among the leading causes of death worldwide. One of the most difficult challenges is cancer stem cell eradication. To further understand the complexity of tumor microenvironment (TME), a panel of cell surface marker for cancer cell differentiation status, including CD44 for stemness and CD54, PD-L1 and MHC class I for more differentiated cells was established. Moreover, it was shown that stem-like cancer cells are more susceptible to NK cell mediated cytotoxicity and can be differentiated by IFN-γ and TNF-α secreted by NK cells, resulting in higher sensitivity to chemotherapeutic treatment in vitro and less tumor growth in vivo. However, it was shown that the number and functions of NK cells are less in cancer patients, which may contribute to the recurrence of this refractory disease. Thus, a novel strategy of expanding the numbers and enhancing the functions of NK cells was developed for NK immunotherapy by utilizing feeder cells, osteoclasts, and sonicated probiotic bacteria (sAJ2). These expanded NK cells, called super-charged NK cells, possess extremely potent cytotoxicity and cytokine secretion abilities. To verify the therapeutic potential of these super-charged NK cells, a tumor-bearing humanized BLT-mouse model was used. Mice that received super-charged NK cells as immunotherapy showed less tumor growth accompanied with higher IFN-γ levels in sera and tumor tissue. In sum, NK cells are essential in halting the progression of tumor by mediating direct cytotoxicity to cancer stem-like cells and cytokine secretion to drive the differentiation of cancer cells in the TME, which may serve to be the key in successful combination therapy strategies.

Super-charged NK cells preferentially select for CD8+ T cells and promote their expansion by increased targeting of CD4+ T cells.

Meng-Wei Ko*

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Natural killer (NK) cells play crucial roles in halting the progression of cancer through selectively lysing and differentiating cancer stem cells via direct cytotoxicity and IFN-γ and TNF-α secretion, respectively. It has been shown that the numbers and functions of NK cells are significantly lower in cancer patients. As such, we have previously established a methodology to expand and enhance the functions of NK cells through osteoclasts and probiotic bacteria. Such expanded NK cells, coined as "super-charged" NK cells (sNK), possess great therapeutic potential. Several techniques were employed to comprehensively understand the differences between primary and sNK cells. By using proteomics analysis, ELISpot, single cell secretome analysis, and single cell RNA sequencing analysis, we established that higher levels of IFN-γ and TNF-α were consistently seen in sNK cells. In addition, more polyfunctional NK cells were seen in sNK cells. However, sNK cells were shown to have a higher abundance of granzyme B but lower amounts of perforin in proteomics analysis, whereas single cell RNA sequencing yielded lower expression of granzyme B and higher expression of perforin in super-charged NK cells, when compared to primary NK cells. Discordant results between protein abundance and RNA expression in cytotoxic factors may be attributed to protein stability and/or post-transcriptional modification of the mRNA or the natural divergence of bulk and single cell analysis. Overall, our study established key differences between super-charged NK cells and those of primary NK cells, which could be directly correlated with the functional capabilities of these cells. In a long-term expansion of super-charged NK cells, we observed the expansion of small subpopulation of contaminating $CD8^+$ T cells. Super-charged NK cells showed the ability to lyse $CD4^+$ T cells but not $CD8^+$ T cells in target cell visualization assay (TVA). Moreover, super-charged NK-expanded $CD8^+$ T cells showed the highest IFN-γ secretion, as well as other important cytokines among other tested groups. Increased CD8+ T cells and an increase in IFN-γ secretion, as well as other important cytokines, were observed in oral tumor-bearing hu-BLT mice. Therefore, NK cells are important in the selection and expansion of $CD8^+$ T cells.