IRIC’S VIRTUAL SYMPOSIUM 2021:
GPCRs in oncology and immuno-oncology
This report summarizes three presentations made at the “IRIC’s virtual Symposium 2021: GPCRs in oncology and immuno-oncology”, held on June 4, 2021. Chaired by Michel Bouvier, CEO of the Institute for Research in Immunology and Cancer (IRIC), and sponsored by Domain Therapeutics, this virtual symposium highlighted some of the latest discoveries and techniques leveraging the potential of GPCRs in oncology and immuno-oncology.

Professor Bouvier introduced the topic and reminded participants that with approximately one-third of marketed drugs targeting GPCRs, the potential of this family of receptors as therapeutic targets is unmatched. However, their involvement and drug discovery potential in oncology and immuno-oncology is somewhat new in spite of the fact that there has been for some time increasing evidence of pluridimensional roles of GPCRs in cancer. Most notably, GPCRs and G proteins are frequently mutated and their expression is dysregulated in many cancers. Knowing that GPCRs are eminently druggable targets, there is a clear untapped potential for the development of GPCR-targeting agents in oncology. This is the topic of this timely virtual symposium and of three presentations by guest speakers who have made significant contributions to this field.
Professor Smit highlighted the fact that many of the GPCRs highly expressed in tumors, are chemokine receptors. In the context of cancer, her group with collaborators in the ONCORNET consortium has focused on the CXCR4/ACKR3/CXCL12 axis and its role in promoting the pro-tumorigenic and immuno-suppressive tumor microenvironment (TME). CXCR4 is a typical Gi-coupled GPCR expressed in almost all tumor types. It binds the ligand CXCL12 (also known as stromal-cell derived factor-1 or SDF-1). The atypical chemokine receptor, ACKR3 (formerly known as CXCR7), was also shown to be implicated in tumorigenesis, particularly in aggressive tumors types. ACKR3 also binds CXCL12 and is believe to scavenge the ligand away from CXCR4 thus modulating its function.

One of their approaches focuses on the use of nanobodies, the smallest antigen binding antibody fragment, consisting of a single monomeric variable domain. They are highly selective and very stable. Their small size (10-15 kDa) allows them to penetrate tissues, and they often recognize conformational epitopes. Professor Smit’s group identified two nanobodies (238D2, 238D4) specific for CXCR4 and showed that they bind extracellular receptor’s loops and displace CXCL12. Combining these two nanobodies created a bispecific nanobody with high affinity for the receptor that acts as a potent inverse agonist and that can inhibit CXCL12 induced chemotaxis. They also showed, in a leukemia model, that by linking the nanobody moieties to Fc chains (Nb-Fc), one could restore the immune function (interaction with NK cells) and induce antibody-dependent cell cytotoxicity. Professor Smit’s group also identified nanobodies targeting ACKR3 and displacing CXCL12. The potency of these nanobodies was demonstrated in a xenograft model of ACKR3-expressing head and neck cancer, where injection of trivalent nanobodies significantly impaired tumor formation and reduced angiogenesis.

Thanks to a large extent to Dr. Smit’s pioneering work, it is also known that viruses can hijack the chemokine receptor system. Professor Smit’s lab is interested in HCMV that encodes four GPCRs resembling chemokine receptors. 50-80% of the human population is HCMV positive but the virus is mostly present in a latent form. Interestingly, HCMV proteins have been detected in a number of tumors (glioblastoma, breast, colon, prostate cancers). Dr. Smit’s group focused on the HCMV encoded US28 GPCR. It shows 30% identity to CCR1 and CX3CR1 (fractalkine receptor) and binds a
variety of chemokines. It is a functional GPCR showing constitutive and ligand-dependent signaling. Early studies showed that in transfected or infected cells, increased US28 levels led to increased InsP production (a Gq signaling read-out) and increased NFκB, leading in turn to COX2 expression and increased VEGF secretion. Fibroblasts transfected with US28 become highly proliferative, form foci and when injected in nude mice cause tumor formation. Furthermore, knock-in mice expressing US28 in the intestine develop intestinal neoplasia. Most importantly, US28 could be detected in human glioblastoma tissue, especially in vascularized areas, but not in healthy tissue. In the lab, spheroid growth of U251 glioblastoma cells and VEGF secretion were significantly increased following infection by wild-type HCMV or induced expression of US28. In an intracranial orthotopic xenograft model, US28 expressing glioblastoma cells caused early onset of tumor formation. Conversely, a bivalent nanobody (VUN100bv) acting as an inverse agonist at US28 impaired spheroid growth and VEGF secretion of US28 expressing glioblastoma cells. In the intracranial tumor model, treatment with a trivalent nanobody against US28 showed decreased and delayed tumor formation.

Intrabodies (intracellular binding nanobodies) against US28, binding the ligand free receptor were also identified. Using a BioID enzyme-coupled US28 construct, they showed that Gq was highly biotinylated in this context. The amount of biotinylated Gq was reduced in the presence of VUN103 indicating that the intrabody disrupted the US28-Gq interaction. This was confirmed by a BRET-based reporter assay. US28 expressing cells exposed to VUN103 showed significantly reduced spheroid growth in culture, supporting the notion that the receptor oncogenic activity involves Gq signaling.

Another aspect of US28 biology was also recently investigated. Dr. Smit’s group observed that secreted exosomes are highly enriched in GPCRs, including US28 and ACKR3. Using tagged US28 constructs (HA or pHluorin) in glioblastoma cells, they could show that US28 is detected in intracellular multivesicular bodies and present on the surface of released exosomes from both transfected and HCMV infected cells. One functional consequence may be scavenging of chemokines by these exosomes, which may contribute to immune evasion. Indeed, Dr. Smit showed that US28 expressing exosomes bind CX3CL1 (fractalkine) and that their presence reduces signaling by the CX3CR1 receptor.

All together, the data presented convincingly show that chemokine receptors (including viral chemokine receptors) play multiple roles in immuno-oncology, that nanobodies are powerful experimental tools to address these roles and that they show potential as therapeutics.
Dr. Leroy provided an overview of different approaches used at Domain Therapeutics and other biotechnology companies to discover GPCR antibodies (Abs) for therapeutic applications. GPCRs have proved challenging targets for the discovery of therapeutic Abs but emerging technologies provide new options and dramatically improve the probability of success.

Phage-display screening of recombinant cell lines is one of the most promising approaches with a number of companies providing services (i.e. Distributed Bio, Mabqi, Proteogenix). It is based on the “panning” of libraries displaying millions of different antibodies on phage, using a cell line transfected with the target of interest fused to GFP. After depletion of non-specific binders and incubation, GFP positive cells are sorted by FACS and phages binding to antigens displayed on the surface of the cells are eluted and amplified. The phages of interest are sequenced and used to generate IgG Abs.

Another approach is to use focused libraries for peptidic GPCRs. For instance, TWIST BIOPHARMA has generated synthetic libraries based on the sequences of binding sites between ligands and receptors, specific extracellular loops, and binding sites for already existing Abs. These diversity libraries are displayed on phage and used to screen for new binders with receptor agonists or antagonists activities.
The screening of hybridomas remains a powerful approach that can be performed in high-throughput format (i.e. MIMAbs, SynAbs). However, in spite of presence of high affinity Abs in the serum of immunized mice, it often remains impossible to identify the producing B cell using this approach. New droplet microfluidic technology (i.e. VELABS) provides a solution. In this approach, single B cells are encapsulated in droplets with a fluorescent reporter signal for GPCR activity. Flow cytometry allows the rapid sorting of hundreds of thousands of B cells and selection of individual B cells producing Abs that activate the GPCR of interest. Finally another microfluidic approaches (i.e. ABCELLERA) allows HTS and multiplexing of single B cell screening. The small volume of microfluidic chambers (1 nl) enables secretion assays of individual B cells in presence of a target-expressing cell associated with microscopy-based imaging that supports a wide array of screening assays. Parallel screening with multiple devises allows throughput of >1M cells per day.

Yeast selection screening is another powerful approach (ie. ABALONE). It is based on the expression in yeast of a GPCR of interest that induces a growth signal upon activation. Billions of yeast cells are then transformed with an Abs library such that each yeast cell expresses a single Ab. In culture, only cells with a functional Ab agonist for the GPCR will grow (a reverse system can also allow selection of antagonists). Single-cell or Next-Gen sequencing of surviving cells provides the sequence of candidate functional Abs.

In summary, one can foresee a bright future for the development of functional Abs against GPCRs and immuno-oncology will soon benefit from these new devices/tools.
Dr. Gutkind, a pioneer of the field of GPCRs in oncology, highlighted the fact that it became clear only in the early 1990s that GPCRs could act as ligand dependent oncogenes and that mutant GPCRs and G proteins could have oncogenic properties. Large cancer genome sequencing projects later found that roughly 20% of all cancers carry GPCRs or G proteins mutations, including mutational hotspots leading to constitutively active $G_\alpha$ proteins. A recent re-analysis of all GPCRs mutated in cancer (the Onco-GPCRome) found that the vast majority of mutated GPCRs are found in gastrointestinal tumors. Mapping these mutations on the canonical structure of GPCRs, showed that most cluster around a very small subset of structural motifs and that they result mostly in gain of function in Gs-coupled GPCRs and loss of function in Gi-coupled GPCRs that activate and inhibit adenylyl cyclase, respectively. This fits with the observation that GNAS (the gene encoding G$_{\alpha_S}$) is frequently mutated in a number of tumors.

Dr. Gutkind then focused on the role of G$_{\alpha_Q}$ and Gq-coupled GPCRs in uveal melanoma (UM), a tumor originating in the pigmented cells of the eye (iris, ciliary body and choroid). There are about 2,500 new cases per year with a 5 yr survival rate of 50% as roughly half of UM patients develop liver metastasis and the majority of metastatic UM (mUM) fail to respond to treatment. Unlike skin melanoma, BRAF mutations are rare in UM but 92% of cases have GNAQ (G$_{\alpha_q}$) mutations (compared to 4% in skin melanoma). A recent analysis of these driver mutations showed that the vast majority affect a single residue, Q209, with fewer mutations affecting R183 and that they result in constitutively active G$_{\alpha_q}$ that activate phospholipase C (PLC). However, attempts to block the canonical Gq/11-PLC-ERK pathway has offered no clinical benefits.

To better understand the pathways involved in the oncogenic role of the activated Gq, Dr. Gutkind and collaborators performed a genome-wide RNAi screen in Drosophila cells, using AP-1 (a downstream target of G$_{\alpha_Q}$) as a readout. Unexpectedly, the screen identified components of the TRIO and Hippo pathways. TRIO is a nucleotide exchange factor that activates the GTPase Rho. They found that when Gq is activated, TRIO is recruited to the membrane and activated in a two step process that involves a very high affinity interaction with Gq.
Interestingly TRIO is overexpressed in UM and knocking it down in a culture system, reduces tumor growth without affecting PLC or ERK activation. The Hippo pathway on the other hand is a tumor suppressor pathway. Activation of the Hippo kinase cascade leads to phosphorylation of a co-activator called YAP (or TAZ) and its exclusion from the nucleus, preventing its binding to transcription factors of the TEAD family, which are important regulator of cell proliferation. In an animal model or in human UM, Gαq can induce the Hippo pathway (as shown by nuclear accumulation of YAP) and knocking down YAP reduces tumor growth in a patient derived xenograft model of UM tumors. Treatment with verteporfin, a drug that disrupts the YAP-TEAD interaction, also reduces tumor growth in this model. Unfortunately, this drug cannot be used for long-term treatments because of toxicity and while other YAP-TEAD inhibitors are being developed, none has yet been approved for UM.

In order to find novel ways to target this pathway, Dr. Gutkind and his collaborators developed a computational pipeline to identify synthetic lethal interactions with GNAQ. This in silico approach identified only 5 genes of interest. The top one was PTK2, a gene encoding the focal adhesion kinase (FAK). It is amplified and overexpressed in UM, where expression correlates tightly with poor clinical outcomes. UM cell lines and tumors were shown to be very sensitive to a number of approved FAK inhibitors (FAKi). In both cases, YAP was excluded from the nucleus in FAKi treated cells. Based on these observations, a multicenter clinical trial using FAKi in mUM was recently initiated.

When using targeted therapies in oncology, one often observe the activation of compensatory mechanisms leading to drug resistance and relapse. This motivated the Gutking group to look for other molecules that when blocked or inhibited would enhance the response to FAKi in the context of Gαq activation. They performed a CRISPR/Cas9 screen in UM cells, to identify synthetic lethal interactions with FAKi. The screen focused on kinases and identified targets that are found mostly in the canonical Gq/PLC pathway (PKC, components of the ERK pathway, etc.). They confirmed that ERKi and FAKi acted in a synergistic manner, and in combination they rapidly induced apoptosis of UM cells. The same synergy was seen in a tumor model where combination treatment led to very significant decrease in tumor size, reduced proliferation and induced cell death. Furthermore, they developed a mouse model for UM related liver metastasis and showed that combination treatment was effective in decreasing tumor burden and increasing survival. Based on these observations, a new multicenter clinical trial is now underway to test this signal-transduction multimodal precision therapy approach.

Dr. Gutkind ended by reminding the audience that abnormal GPCRs signaling in absence of mutations in GPCRs or G proteins genes is also implicated in a number of oncocrine networks and that novel ways of targeting the chemokine GPCRs oncocrine networks has the potential to enhance the response to immunotherapies.
ABOUT THE INSTITUTE FOR RESEARCH IN IMMUNOLOGY AND CANCER (IRIC)

An ultra-modern research hub and training centre located in the heart of the Université de Montréal, the Institute for Research in Immunology and Cancer of the Université de Montréal was created in 2003 to shed light on the mechanisms of cancer and discover new, more effective therapies to counter this disease. The IRIC operates according to a model that is unique in Canada. Its innovative approach to research has already led to discoveries that will, over the coming years, have a significant impact on the fight against cancer.

ABOUT DOMAIN THERAPEUTICS

Domain Therapeutics is a french-canadian biopharmaceutical company committed to the discovery and development of innovative treatments for immuno-oncology tackling GPCR mediated-immunosuppression. Domain identifies and develops candidates modulating GPCRs through innovative approaches and proprietary technologies, such as bioSensAll®.

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