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Meeting Report

Aptamers in Bordeaux 2017: An exceptional *"millésime"*

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ABSTRACT

About 150 participants attended the symposium organised at the *Palais de la Bourse* in Bordeaux, France on September 22–23, 2017. Thirty speakers from all over the world delivered lectures covering selection processes, aptamer chemistry and innovative applications of these powerful tools that display major advantages over antibodies. Beyond the remarkable science presented, lively discussion and fruitful exchange between participants made this meeting a great success. A series of lectures were focused on synthetic biology (riboswitches, new synthetic base pairs, mutated polymerases). Innovative selection procedures including functional screening of oligonucleotide pools were described. Examples of aptasensors for the detection of pathogens were reported. The potential of aptamers for the diagnostic and the treatment of diseases was also presented. Brief summaries of the lectures presented during the symposium are given in this report. The third edition of this symposium will take place in Boulder, Colorado in Summer 2018 (information available at http://www.aptamers-in-bordeaux.com/).

1. Introduction - aim and scope of the symposium

A bit more than one year after the first edition of "Aptamers in Bordeaux" [1] aptamer experts from all over the world aggregated again in the reknown city of Bordeaux. The many facettes relevant to this promising class of oligonucleotides were covered, from "aptamerology" -the way to select, characterize and improve aptamers- to the most advanced applications in human health. Following a session dedicated to artificial riboswitches we heard on the one hand about the selection of aptamers that included modified bases, making the aptamer repertoire more and more diverse, and on the other hand about a bunch of processes derived from regular SELEX: SELkiss, Aptaswift, LIGS …. Characteristics and properties of aptamers raised against a very diverse range of targets (metal cations, opioids, influenza hemagglutinin, histones, factor IXa, etc …) were described. These aptamers and others were converted into biotechnological tools or integrated into devices for analytical or diagnostic purposes. Therapeutic applications were also described for cardiac surgery or for the treatment of age related macular degeneracy, multiple organ degeneracy, non-Hodgkin lymphoma or glioblastoma. Remarkably, 28 countries were represented this year by at least one participant. Thirty PhD students or post-docs competed for the three poster awards sponsored by "Novaptech" and by "Biomedicines". In addition about 40 participants from 26 biotech or pharma companies attended the symposium, a marked increase compared to the 2016 meeting. Larry Gold might be right in writing in his abstract that *"aptamers […] are approaching their moment in the sun"*.

2. Riboswitches and synthetic biology

The meeting started with talks dealing with the use of aptamers in synthetic biology and more specifically with the design of novel riboswitches to artificially regulate gene expression. Indeed engineered regulation of genetic circuits is a very exciting and active field. Among the regulatory elements for circuit design, riboswitches are prominent and elegant examples. Riboswitches consist in RNA motifs comprising a so-called "aptamer domain". The binding of a small molecule to this domain results in a conformational change which then determines the output of the downstream "expression platform". **Prof Beatrix Suess** (Darmstadt

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University, Darmstadt, Germany) described the use of capture SELEX [1] to select aptamers targeting small molecules such as antibiotics that can be used for riboswitch design. RNA-aptamer libraries were then screened *in vivo* in yeast, with a method based on fluorescence readout, to identify sequences that control translation in a ligand-dependent fashion. In the second part of her talk, she introduced new data about the design of a light responsive riboswitch. **Dr Sven Feindeiss** (Leipzig University, Leipzig, Germany) took another approach to engineer artificial riboswitches. He presented a new technique based on *in silico* prediction to transform a known aptamer sequence into a functional riboswitch. As a proof of concept, he applied his bioinformatic tool to transform the theophylline aptamer into a synthetic riboswitch able to control gene expression at the transcriptional level in *Escherichia coli*. Furthermore, he showed that these elements can be combined in tandem or tridem arrangements, which strongly reduced the background activity. He then concluded by discussing various limitations of *in silico* approaches and more specifically what could be improved to increase the fold change of regulation in response to the ligand. **Dr Alexander Taylor** (MRC, Cambridge, UK) described a series of synthetic alternatives to DNA composed of non-natural building blocks, termed 'xeno nucleic acids' (XNA), to evolve functional aptamers (XNA aptamers) and enzymes (« XNAzymes ») such as endonucleases and DNA ligases [2]. He also presented a series of applications for these non-natural compounds, for example the development of new material and in particular their use in nucleic acid nanotechnology, such as self assembly of 3D-structures [3]. Finally his presentation highlighted the great potential for XNAs, including resistance to nucleases, to provide novel tools in aptamer research as these modified nucleic acid analogs can be manipulated by recombinant enzymes such XNA polymerases or XNA-dependent DNA polymerases. A selected FANA aptamer was active against the Ebola virus. **Prof Steven Benner** (Foundation for Applied Molecular Biology, Alachua FL, USA) gave a fascinating talk on the development of artificially expanded genetic information systems (AEGIS) [4]. Due to the limited number of functional groups from the four standard nucleotides, standard RNA/DNA (collectively named NA) cannot yield a rich enough diversity for binding and catalysis. Moreover, the low information density of the four letter genetic system appears to diminish the performance of NA molecules due to ambiguous/alternative inactive folding. In order to overcome these limitations of natural NA, both modified NA bases with additional functional groups and new base pairs were synthesized in Benner's lab over the last 25 years. In parallel, enzymes to replicate, transcribe, reverse-transcribe AEGIS oligonucleotides were developed as well as molecular tools for sequencing them. Having every tool in hands, SELEX with AEGIS libraries was carried out against several targets. MDA-MD-231 breast cancer cell line, the toxic form of anthrax protective antigen [5] and Glypican 3 expressing liver cancer cells were targeted. The results clearly demonstrate that incorporating AEGIS nucleotides helps both improving the specificity of the aptamers and decreasing the number of selection rounds needed. Moreover, ambiguous folding is reduced leading to a better functionality of these AEGIS aptamers.

Dr Juewen Liu (University of Waterloo, Waterloo, Canada) presented an interesting work on the selection of metal-binding aptamers, a challenging task as metal ions cannot be immobilized without impacting their coordination potential. For circumventing this limitation, Dr Liu made use of catalytic DNA selection. Using this process, he was able to select after a few rounds the first RNA-cleaving DNAzyme using cerium, a trivalent lanthanide metal ion, as the sole metal co-factor. Interestingly, this lanthanide-dependent DNAzyme needs also Na^+ to work. Using 2-aminopurine as a label, Na^+ concentration was monitored [6]. Moreover, a phosphorothioate modification at the cleavage site allows this lanthanide-dependent DNAzyme to detect specifically thiophilic metals. In addition to the lanthanide-dependent DNAzyme story, Juewen Liu also presented a catalytic DNA selection process that leads to the obtention of a silver DNAzyme with an impressive detection limit of 25 nM [7]. He is developping aptamers against a wide range of lanthanides, performing only 5 SELEX rounds.

3. Selection methodology and chemically modified aptamers

Researchers at SomaLogic, demonstrated the benefit of introducing functional groups mimicking amino acid side chains at one of the four nucleic acid bases (uridine) for the obtention of SOMAmers (Slow Off- rate Modified Aptamers) [8]. **Prof Larry Gold** (Somalogic, Inc., Boulder, CO, USA; see Fig. 1) indicated that 5000 somamers were identified and that the structure of three crystallized aptamer-protein complexes was solved. Modified nucleotide side chains create generally intramolecular motifs (benzyl zipper, hydrophobic cluster) [9]. The association with the target protein is not driven by electrostatic interactions. Surprisingly somamers are poorly structured and show few, even no Watson Crick pairs. Larry Gold then elaborated on globularity, a characteristic of enzymes and a challenge for catalytic RNAs. He described the properties of short hairpins containing benzyl-dU in their loop that turn out to be extremely stable, even more than the tetraloops GNRA and UNCG. **Dr Bharat Gawande** (Somalogic, Inc., Boulder, CO, USA) presented a new development of the SOMAmer selection. The possible benefit of the introduction of two different modifications on two bases was questioned. Several selections were performed against proprotein convertase subtilisin/kexin type 9 (PCSK9) with libraries containing modifications at the C5 position of either dC or dU or both dC and dU [10]. It was demonstrated that some doubly modified SOMAmers (hydrophobic side chain on cytidine combined with a tyrosine-like side chain on uridine) resulted in a high affinity (Kd lower than nanomolar). Moreover, two modified SOMAmers exhibited a large target epitope coverage thus improving sandwich pair identification. Among the sandwich pairs tested, the best exhibited an affinity in the picomolar range.

Several talks were dedicated to new selection methodologies. Light up aptamers are aptamers that, upon binding, dramatically increase the fluorescence quantum yield of fluorogenic molecules that are non fluorescent in the free state. However a very limited fraction of aptamers to fluorogens translate their association into fluorescence emission. **Dr. Michael Ryckelynck** (University of Strasbourg, Strasbourg, France) described a powerful functional screening based on microfluidic-assisted compartmentalization for the selection of RNA light up aptamers. Each candidate is individually encapsulated into picoliter water-in-oil droplet where it is PCR-amplified and transcribed. After addition of the fluorogen molecule into each droplet, light-up RNA aptamers are collected in a high throughput manner (up to several millions variants are analyzed per day). Mutants of iSpinach and Mango light up aptamers with improved fluorescent properties were uncovered using this approach [11]. Moreover, an efficient iSpinach-derived biosensor for theophylline detection was obtained thus demonstrating the efficiency of this approach for the selection of light up aptamers. Microfluidic-assisted selection constitutes an efficient and quick method for the identification of functional aptamers as far as a fluorescent reporter is available. However pre-selection is required as the method does not allow handling the complexity of 10^{14} candidates in starting pools routinely used for selection. This is actually also the case for the method called APTASWIFT described by **Dr Christin Rath** (University Freiburg, Freiburg, Germany) based on affinity screening without prior need for cloning or sequencing.

Fig. 1. Prof Larry Gold (left) and Dr Jean-Jacques Toulmé (right) started preparing the 2018 Aptamer symposium during the Gala dinner, in *La Cité du Vin* in Bordeaux.

The candidate population is distributed in a cavity chip at less than one DNA copy per cavity. A patented solid-phase PCR allows the production of a DNA pixel from each cavity on a glass surface [12] thus generating a microarray of the selected population. Binding parameters of 4000 individual aptamers are measured in real-time and in a label-free process via imaging reflectometric interference [13]. The best aptamers are then harvested from the microarray via photo-cleavage and sequenced. **Dr Prabodhika Mallikaratchy** (The City University of New York, New York, NY, USA) presented a variant of cell SELEX termed Ligand-Guided-Selection (LIGS) for the selection of aptamers against cell surface protein in their native conformation [14]. After a few rounds of standard cell SELEX, a pre-enriched pool of aptamers raised against various aptatopes is introduced into a competitive process. In order to collect aptamers to a given cell surface protein, a known binder of this particular target is introduced thus outcompeting the desired aptamers. Using the same pre-enriched pool, a number of cell-surface targeting aptamers can be obtained through this strategy as far as competitive binders for other targets are available. A proof of concept was described with aptamers exhibiting a Kd in the low nanomolar range selected against membrane bound IgM (mIgM) expressed on B-cells [15] and cluster of differentiation 3 (CD3) expressed on T-cells.

Capillary electrophoresis SELEX (CE-SELEX) allows performing the selection of aptamers with a reduced number of selection rounds, requires a limited amount of material and circumvents the requirement of target immobilization. This is an interesting technique even though the complexity of the pools that can be handled is reduced compared to standard selection and is restricted to targets of large size [16]. **Dr Corinne Ravelet** (Université de Grenoble-Alpes, Grenoble, France) illustrated the potential of CE-SELEX with two examples. Firstly, she described the selection of aptamers to the alphaC-conotoxin PrXA. The winning candidate identified after only four selection rounds, characterized by a Kd of about 100 nM was shown to neutralize the toxin in mice. Secondly, she compared the outcome of classical CE-SELEX and non-CE-SELEX in which the amplification step at the end of every SELEX round was omitted. An enriched population displaying a Kd of about 40 nM for the Tau protein, a biomarker of Alzheimer disease, was obtained through non-CE-SELEX whereas the classical CE-SELEX failed. **Prof Maxim Berezovski** (University of Ottawa, Canada) described an original application of aptamers for the purification of live cells i.e. separate them from undesired material in samples such as cord blood or bone marrow. The release of affinity-captured cells by antibodies rely on the competition with either a peptide antigen or with an antibody to a Fab fragment. In the aptamer-facilitated cell purification the release can take advantage of peculiarities of oligonucleotide such as the degradation of the aptamer by nucleases or its conformational change induced by a change in the buffer conditions. In the technique described by M. Berezovski cell SELEX is carried out in the presence of Ca^{2+} or Mg2+ ions. The same ionic conditions that allow for an active structure are used for capturing cells of interest by the selected aptamers. EDTA or EGTA is then added; the removal of divalent cations is supposed to lead to the loss of the active structure ant to the dissociation of aptamer-cell complexes. This procedure was proven to be efficient for isolating EDTA-switchable aptamers to $CD14^{+}$ monocytes from human blood by FACS. These aptamers were subsequently successfully used for purifying monocytes.

High-Throughput Sequencing (HTS) that enables the identification of millions of DNA molecules is widely used for monitoring the evolution of *in vitro* selection and for extracting aptamer sequences from selected pools. The analysis of big data generated by this technique requires developing new *in silico* tools. **Dr. Frédéric Ducongé** (CEA-University Paris-Saclay, Fontenay aux Roses, France) described PATTERNITY-seq, a bioinformatic tool for HTS analysis of successive SELEX rounds [17]. PATTERNITY-seq measures the frequency of every sequence as well as their evolution from round to round (mutation, amplification, extinction). It generates sequence clustering in families from which a phylogenetic diagram is built. Shared predicted structural motifs between different sequences or families can also be identified. PATTERNITY-seq helps to better understand the impact of the selection pressure and to identify best aptamers that can be under-represented in the selected pool.

4. Aptamers as diagnostic and analytical tools

Native electrospray ionization mass spectrometry (ESI-MS) preserves non-covalent interactions. This label-free technique allows getting access to the stoichiometry of complexes and to Kd values. Furthermore, ESI-MS coupled to ion mobility spectrometry (IMS) provides information about the binding mode and the shape of the molecules [18]. **Stefano Piccolo** (University of Bordeaux, Inserm, CNRS, Bordeaux, France) demonstrated the usefulness of ESI-MS coupled to IMS for the characterization of RNA/ligand complexes. He described his current investigation on cocaine and tobramycin aptamers that are known to change conformation upon binding to their cognate target. Several cases of severe and even fatal food-associated anaphylaxis due to lupin -which is increasingly used in a variety of food products- have been reported. An aptamer termed β-CBA I was raised against the lupin subunit β-conglutin that was identified as the allergen [19]. **Dr Ciara K. O'Sullivan** (Universitat Rovira I Virgili, Tarragona, Spain) described the characterization and the improvement of the β-CBA I aptamer for developping biosensors. The truncation of the parent aptamer down to eleven nucleotides resulted in an increased affinity by two orders of magnitude. CD and NMR structural studies suggested a dimeric G- quadruplex folding. 5′ or 3′ end terminal modification impacted differently the structure and the binding properties of this aptamer. This G-quadruplex forming aptamer displays a high specificity for β-conglutin and shows no cross-reactivity to other lupin conglutins (α-, δ-, γ-conglutins) and gliadin. The robustness of the aptamer was validated since it led to a positive enzyme-linked oligonucleotide assay [20]. Kissing interactions result from the formation of Watson-Crick base pairs between complementary loops of RNA hairpins. **Dr Jean-Jacques Toulmé** (University of Bordeaux, Bordeaux, France and Novaptech, Pessac, France; see Fig. 1) reported on the design of kissing complex-based aptasensors in which one of the partners is a structure switching aptamer. Aptaswitches specific for various purine derivatives were described allowing their specific detection through diverse modalities (surface plasmon resonance, fluorescence anisotropy, colorimetry) [21,22]. Interestingly such aptasensors remain functional in organic solvents such as methanol or acetonitrile making them interesting for analytical detection of organic compounds. The repertoire of kissing motifs was extended from RNA-RNA to DNA-DNA complexes. Kissing interactions allow the selection of functional aptaswitches against free molecules in solution through a procedure named SELKISS. **Dr George W. Jackson** (Base Pair Bio-Technologies, Pearland, TX, USA) reported on aptamers developed by the company to a number of opioids and to their primary urinary metabolites. In collaboration with Vanderbilt University, these aptamers were incorporated into assays based on backscattering interferometry [23] allowing subnanomolar detection of opioids and their metabolites. In a separate project related to drugs of abuse, the authors have recently developed high affinity, highly selective structure-switching aptamers to levamisole, originally an antiparasitic drug frequently used as a cutting agent for illicit cocaine. Using these newly developed aptamers, a rapid lateral flow test for levamisole in urine was designed.

Aptamers are promising candidates for point of care diagnostic assays. Two talks addressed this particular aspect for the detection of human pathogens. **Dr Julian A. Tanner** (University of Hong Kong, Hong Kong SAR, China) presented progresses about a malaria detection assay based on an aptamer with a Kd of 20–60 nM for the *Plasmodium* lactate deshydrogenase used as a biomarker [24]. This aptamer was successfully used in an Aptamer Tethered Enzyme Capture (APTEC) assay that proved competitive in sensitivity and specificity with the very best antibody-based methods using clinical samples. It shows a high specificity for *P. falciparum* but does not allow the detection of *P. vivax*. Integration of APTEC into a 3D printed millifluidic device enabled a lowcost equipment-free approach for malaria diagnosis [25]. Ongoing research deals with the integration of aptamers into DNA nanostructures for the controlled delivery of medicines, for instance. In the case of the influenza virus it is of interest to develop assay systems specifically detecting subtypes. **Dr Junyoung Kwon** (University of Science and Technology, Pohang, Korea) described the selection procedure alternating positive selection rounds against the ecto- domain of hemagglutinin 3 (HA3) and a surrogate baculovirus containing the full-length HA3 protein of influenza virus. In addition highly stringent counter-selection against hemagglutinins of other influenza virus strains increased the specificity. The replacement of standard dUTP by benzyl-dUTP allowed the selection of a pair of aptamers with nanomolar affinity for HA3. A lateral flow assay was subsequently developed that specifically detected the influenza A virus subtype H3N2, leading to the most sensitive diagnostic system to date. The new aptamer-based diagnostic system of influenza was much more sensitive than a commercially available subtype-specific diagnostic system of influenza virus composed of antibodies.

5. Pre-clinical and clinical application of aptamers

Dr Paloma Giangrande (University of Iowa, Iowa City, IA, USA) presented two stories: the design of a circulating tumor cell (CTC)-based diagnostic assay and the development of tools of interest for multiple organ dysfonction syndrome (MODS). The detection of CTC–derived nucleases that are over-represented in the circulation of cancer patients is based on the digestion of an oligonucleotide molecular beacon conjugated to a FAM fluorophore and to a fluorescence quencher [26]. The assay detected higher nuclease activity in the blood of breast cancer patients than in healthy ones [27]. The second point of her presentation dealt with the use of RNA aptamers against circulating histones H3 and H4. Many survivors of major trauma and severe tissue injury develop secondary organ failure as a consequence of their primary injury, a situation known as MODS. MODS is mediated in part by the cellular release of histones. RNA aptamers were selected that bind with a low nM-pM affinity and a high specificity to H3 and H4, and reversed histone-induced cytotoxicity both *in vitro* (lung-derived endothelial and epithelial cells) and *in vivo* in a mouse model of MODS. Biomedical applications of magnetic nanoparticles combined with magnetic field proved to be useful in cancer therapy. **Dr Kichkailo-Zamay** (Krasnoyarsk State Medical University, Krasnoyark, Russia) described gold-coated magnetic nanoparticles (GMNPs) grafted with a DNA aptamer targeted to mouse Ehrlich carcinoma fibronectin. Exposure to an alternating magnetic field of 50 Hz at the tumor site induced nanoparticle oscillation, pulling the fibronectin to the surface of the cell, subsequently leading to apoptosis followed by the necrosis of tumor cells. Such a treatment resulted in a significant decrease of the tumor size after 5 days of the tumor transplantation in a mouse model. Grafted GMNPs do not show hepatoxocity in healthy animals [28]. This technique can also be considered for the microsurgery of tumors or for the treatment of glioblastomas by spray. The selection of aptamers recognizing the surface of tumor cells opens the way to tools for targeted delivery as well as to a new class of anti-cancer drugs poorly explored up to now. **Prof Günter Mayer** (University of Bonn, Bonn, Germany) described an aptamer recognizing non-Hodgkin lymphoma (NHL) B-cells that was selected by FACS-SELEX. The aptamer was then chemically modified (Aptamer Based Affinity Labeling) and used on cultured cells. This aptamer that adopts an unusual quadruplex structure is internalized through clathrin-mediated endocytosis and co-localizes with transferrin [29]. It specifically inhibits

Burkitt's lymphoma cell proliferation and is inactive against normal B-cells. **Dr Nicola Stonehouse** (University of Leeds, Leeds, UK), working on psoriasis and eczema reported that an RNA aptamer can be delivered into skin without the use of lipid-based reagents. The fate of a fluorescently- tagged aptamer was monitored into both dermal and epidermal skin compartments. She reported that a IL-17A-specific aptamer blocked the IL-17 mediated signalling activities in a fibroblast/lymphocyte environment. Further application is to deliver aptamers directly to the site of inflammation. **Prof Weihong Tan** (Hunan University, Changsha, China, and University of Florida, Gainesville, FL, USA) reviewed aptamers that were selected by cell-SELEX in his lab and focused on the use of aptamers for theranostics in particular in cancer research. Eighteen aptamers were tested on cell leukemia from 875 patients before and after chemotherapy treatment. Other applications were mentioned: aptamer-drug conjugates, discovery of a new biomarker by cell-SELEX coupled with mass spectrometry and two-dimension gel electrophoresis. New nucleic acid bases were introduced into aptamers, including photoresponsive pyrimidine derivatives.

A number of cell-internalizing aptamers were previously described for the delivery of siRNAs or small molecules. Glioblastoma (GBM) is the most common primary brain tumor and one of the most lethal cancer sustained by glioblastoma stem-like cells, a small population of stem cells. Due to the role that they play in cancer, miRNAs are of interest either as targets or as drugs. Ideally, anticancer gene therapeutics should be able to specifically target cancer cells and/or tumor associated cells [30]. Dr **Vittorio de Franciscis** (CNR, Naples, Italy) made use of aptamers named GL21.T and Gin4.T specific for the receptor tyrosine kinases Axl and PDGFRβ, respectively, for delivering miRNAs or siRNAs. He described the effect of a siRNA antagonizing the signal transducer and activator of transcription-3 (STAT3) that is known to be a key regulator of the highly aggressive glioblastoma subtype acting as an inhibitor of cell survival. Interestingly the aptamer-siRNA chimera is transported through *an in vitro* model of the blood brain barrier. It was also demonstrated that the selective targeting of tumor cells resulted in the accumulation of the chimera at the tumor site. This opens the way to the targeted delivery of therapeutic oligonucleotides to GBM cells. A similar strategy was presented by **Dr David Porciani** (University of Missouri, Columbia, MO, USA) for the delivery of large RNA payloads to cultured cells. He described an RNA nanostructure assembling RNA modules through a double-stranded connector. The cell-internalizing moeity aptamer targeting the human transferrin receptor was associated to a reporter RNA payload 175-250 nucleotide long, including three-way junction multimeric variants of the Broccoli light up aptamer. This modular nanostructure assembled efficiently in a complex whose functionality was retained. Flow cytometry and confocal microscopy analysis showed that the construct was specifically internalized in Tumor B-cells (but not in T cells) as efficiently as an Alexa 488 labeled siRNA. The intracellular trafficking was monitored showing endosomal escape of the reporter. Such aptamer nanostructures are likely to be of interest for the delivery of large functional RNA like lncRNAS and/or for monitoring the intracellular fate of transcripts.

Reprogramming the ligand specificity of cell membrane receptor is of interest for applications in regenerative medicine. Up to now this is achieved via the genetic engineering of known receptors. Taking advantage of bispecific DNA aptamers **Prof Shinzuke Sando** (University of Tokyo, Tokyo, Japan) described a powerful and promising non-genetic approach for reprogramming the ligand specificity of Met, a member of the receptor tyrosine kinase family that responds to the Hepatic Growth Factor and displays mitogenic and anti-apoptotic effect. The activation of the Met signalling pathway requires the dimerization of the receptor. This was achieved by Prof Sando and his colleagues using the previously identified aptamer SL1 to Met. Indeed SL1 dimer suppresses the apoptosis of hepatocytes [31]. They then conjugated SL1 to a second aptamer in order to promote the dimerization of Met by an external trigger. As a proof of concept they prepared two chimeras in which SL1 was associated to one of the two aptamers TBA15 or THA29 known to recognize two different epitopes on thrombin. The simultaneous addition of the two chimeric aptamers and of thrombin induced Met phosphorylation whereas aptamers alone or thrombin alone did not. Next the PDGF homodimer was used as a trigger together with a bispecific aptamer made of SL1 associated to a previously reported 39 nt long anti-PDGF aptamer. In the presence of PDGF this construct was shown to activate the Met receptor. Interestingly this aptamer-growth factor combination induced the mobility of epithelial cells in culture, indicating that the reprogrammed receptor recapitulated the original biological function. Therefore bispecific aptamers can rewire cellular networks without genetic engineering, a major advantage over antibodies. **Prof Bruce Sullenger** (Duke University, Durham, NC, USA) is working on anticoagulant and anti-thrombotic aptamers and their antidotes [32]. He reported on the use of a controllable factor Xa anticoagulant RNA aptamer to reversibly modulate blood coagulation during cardiopulmonary bypass. This aptamer named 11F7t is able to bind both X and Xa factors but does not occlude the active site. The combination of aptamer and of an active site blocker prevents clotting. The effect is reversed by the addition of the antidote oligonucleotide. Aptamers constitute promising agents for monitoring and controlling blood coagulation. Fibroblast growth factor 2 (FGF2) plays a crucial role in bone remodeling, exhibits a strong angiogenic activity and stimulates the VEGF production. **Pr Yoshikazu Nakamura** (Tokyo University and Ribomics, Tokyo, Japan) reported that RBM-007, a 36 nucleotide long RNA aptamer displaying a Kd of 20 pM for human FGF2, inhibited its binding to each of its four cellular receptors and restored osteoblast differentiation. RBM-007 effectively blocked the bone disruption in mouse and rat models of arthritis and osteoporosis. The interest of RBM-007 was demonstrated in a transgenic mouse model of achondroplasia carrying the fgfr3 mutation that leads to an excess of FGF signalling and shutdown of epiphyseal growth. RBM-007 also showed an anti-choroidal neovascularization effect in mice, i.e. an effect superior or equivalent to Lucentis, an anti-VEGF drug. The injection of this aptamer (1 time; 15 μg/eye) prevents the formation of scar under the retina of rats irradiated 7 weeks before, a lesion that cannot be prevented by anti-VEGF drugs. RBM-007 has a half-life of three days in plasma and shows a good pharmacokinetic profile. Human trials for AMD and Achondroplasia will be initiated in 2018.

6. Conclusion

Three out of fifty posters presented by students and post-doc researchers were selected for awards sponsored by “Novaptech” (http://www.novaptech.com/) and “Biomedicines” (http://www.mdpi.com/journal/biomedicines). The “Novaptech” award was given to Dehui Kong from the University of Georgia (Atlanta, GA, USA) who presented a new method for expanding the chemical functionality of aptamers. Chemically-modified pentanucleotides bearing a unique modification were assembled on a template library by the T4 ligase. This new process named LOOPER for Ligase-catalyzed OligOnucleotide PolymERization was applied to the selection of aptamers to thrombin. The two “Biomedicines” awards were attributed to Irina V. Randrianjatovo-Gbalou (Institut Pasteur, Paris, France) who reported on the enzymatic production of RNA analogs by a DNA polymerase mutant able to incorporate 2′- and sugar-modified nucleotides and Brett Schrand (University of Miami, Miami, FL, USA) who described the use of CpG oligonucleotide-

siRNA conjugates for the vaccination of mice against neoantigens in order to control tumor growth. The Aptamers in Bordeaux symposium provided a very informative and stimulating forum to the participants that will be invited to meet again in Boulder, CO, USA, August 3–4. (See Fig. 1 and http://www.aptamers-in-bordeaux.com/ for information).

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