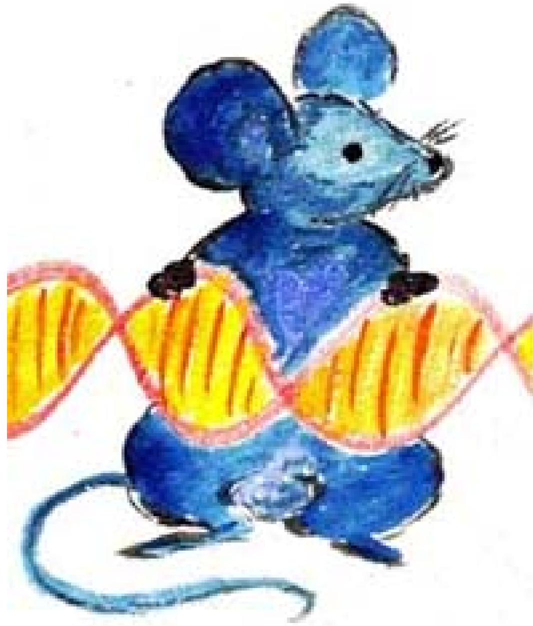

6th Workshop on Innovative Mouse Models, IMM 2011, May 12-13, Leiden, the Netherlands: a meeting report

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On May 12 and 13 2011 the 6th Workshop on Innovative Mouse Models was held in the Leiden University Medical Center, Leiden, the Netherlands. The primary goal of this two-day workshop has always been to bring together a diverse group of scientists interested in advanced genome alteration approaches in the mouse, including key developers of emerging technologies as well as researchers who wish to apply and assess these new approaches. The workshop encourages an in-depth and unvarnished discussion of these technologies and novel developments in a very open and informal way, easy accessable for young and senior researchers (see http://workshop.nki.nl). This year the workshop sessions consisted of:

- Constructs and Genetic modification design
- ES cell derivation and culture
- Induced pluripotent stem cells
- Nucleases
- miRNA
- Applications/Models
- Resources
- Forum discussion

Every session was composed of one or two keynote lectures and some short presentations so the participants were able to hear about the principles, get an overview, and hear about the newest developments by the senior professional, and listen to the junior expert in the field with the latest hands-on experience, either with negative but very interesting results, or with successful developments.

The workshop was kicked off by Francis Stewart, the expert in recombineering and site-specific recombinases. Francis stressed the importance and impact of understanding the mouse genome and compared it with landing a human on the moon or discovering the Higgs boson: all have a big impact on mankind but do cost a lot of money. The different steps to solve are: Understand gene expression pattern, understand cell lineages, target every gene, tag proteins and perform model development. He once again drew the attention to the International Knockout Mouse Consortia and stressed the high throughput and the urgency to always run a Southern of the cells before making a mouse. The International Mouse Phenotyping Consortia are developing also rapidly now. Stewart's focus still is on recombineering using E.coli and BAC constructs, protein tagging and imaging. He showed the latest updates on the liquid system, the use of R6K plasmids to lower the background and the use of nucleofection in human ES cells (leads to silencing of nanog) and the alternative by using piggybac transposons.
Rob Howes was the second keynote speaker in this session and he gave an overview of rAAV-mediated gene targeting in human and animal cells. Rob showed an overview comparison of targeting techniques: transient overexpression, stable overexpression, transient RNAi, stable RNAi, dominant negative overexpression, meganucleases, TALE nucleases, and homologous recombination. And he summarized the major developments in using rAAV-mediated gene targeting since 2005: SEPT (synthetic exon promoter trap) targeting, competing NHEJ, Creating a null allele with rAAV targeting: parental cell line, targeting first allele, removal of resistance gene, targeting second allele, WT virus is a non-pathogenic virus, in recombinant AAV vectors no viral sequences remain, tropism: applicable to mammalian cell types, several cell-surface receptors for AAV, no off-target integrations during rAAV-mediated HR because no introduction of dsbreaks. More information and features can be found at the on- line forum: raavers.org. Personally I found the overview very nice but got lost in the too smooth results and sale promotion at the end of his presentation.
The session was completed with three short presentations covering different uses of constructs and genetic modification design: Conditional by INversion combined with MultiFunctional Allele, humanized mouse genomes and last but not least the Phi31 recombinase-based genomic docking site. This last presentation by Cor Breukel was one of my favourites because Cor showed the experimental ups but also the downs, one of the initial main goals of the workshop, and recognizable for all researchers.

The second session had ES cell derivation and culture as theme and contained two keynote speakers. The first was Jennifer Nichols who started with a nice and didactic overview of early mouse development followed by a historic overview of culturing ES cells, starting with mouse embryonic fibroblasts, shifting to the application of culturing with LIF and finally developing into the use of the small inhibitors- containing medium 3i and 2i. At first we were only able to efficiently develop ES cell lines from the 129 background but blocking the Erk signalling pathway made it possible to develop C57Bl6 ES cell lines. Later on the ground state of ES cells could be shifted by the use of blocking receptors and the use of the 3i medium and 2i medium was a fact. Jennifer also showed that the 2i

medium is not destroying the primitive endoderm but is expanding the epiblast. She prefers to grow cells on LIF, but to use the 2i medium to derive ES cells and she wants to go for development of a 1i medium with the addition of LIF. The growth of ES cells during the derivation is particular: cells tend to form spheres and detach from the bottom. Suggestions were done by the public to change the matrix of the culture dishes from gelatin to laminin, but the best ES cell lines come from the nonadhering cells. The second key note speaker in this session, Ivo Huybers, showed the fast track production of complex tumor models starting from Genetically Engineered Mouse Models (GEMMs). In this approach GEMMs are expanded to non-germline mouse models, using the chimeric animals as first choice models in studying the GM and WT interactions. Derivation of ES cells from these GEMMs gives you GEMM-ESC which in combination with Loss-of- Function and Gain-of-Function vectors will give rise to new combination models without the time consuming breeding of animals and high numbers of surplus animals. This is an efficient creation of cohorts for study. Injection of those GEMM-ESC in morulas, selects for those ESC that do have the potency to contribute to the new generation, providing you with an efficient way of selecting the clones that work and those that don't.

Unfortunately Samer Hussein, key note speaker of the third session, missed his plane and was not able to attend the meeting and tell us about the high level of mutations observed during reprogramming in early passage human iPS cells. His results would have demonstrated the need to understand how the process of reprogramming may undermine the genomic integrity of the cells, and uncovers new questions regarding the DNA stability of reprogrammed cells and the mechanism behind reprogramming –induced mutations. The organisation had looked forward to this topic and hoped for a comparison and discussion about derivation of ES cells and iPS cells, but we hope to have this opportunity in near future. Lieven Haenebalcke made up for the disappointment about the absence of Hussein by presenting an excellent poster and short lecture about the Rosa26-iPS mouse: a novel conditional, inducible and exchangeable resource for studying cellular (de)differentiation. Lieven started with the limitations of the iPS cells: the reprogramming factors Oct 4, Sox2, Klf4 and c-Myc (OSKM) are very potent transcriptional regulators whose misexpression has been implicated in cancer initiation and progression; therefore, an efficient and reliable method for their removal after iPS cell generation is highly desirable. Secondly, the reprogramming factors were initially delivered in somatic cells via retroviral vectors that may disrupt endogenous gene function. The third limitation is the need for efficient ways to differentiate these iPS cells in a lineage-directed manner so that they can be used to repair a given tissue. Haenebalcke et al. developed a double transgenic reprogrammable mouse system based on the doxycycline inducible rtTA-ROSA26 mouse made cell/tissue-specific and inducible. After reprogramming, the four factors can be easily replaced by any cDNA/ORFs of interest via recombinase mediated cassette exchange. For this excellent contribution characterized by a good concept and widely applicable, which could be studied in more detail on the presented poster, Lieven received the poster award. The session was concluded by an application of differentiated iPS cells in human cardiac sodium channelopathy by Richard Davis. The day was closed with drinks and dinner at Restaurant Luxor in the centre of Leiden.

In the fourth session all nucleases had their turn: Ralf Kuhn and Guillermo Montoya both gave an update about the protein scaffolds, Mario Hermann introduced the zinc finger nucleases in the mouse oocytes and Tomas Cermak introduced engineered TAL effector nucleases as new tools for genome editing. All protein scaffolds as homing endonucleases, meganucleases, ZNF nucleases and TALE nucleases are used to target homologous recombination, they all consist of a DNA recognition module and a catalytic module and the trick for all these protein scaffolds is to make them specific enough for the desired GOI, to design them as intelligent cutters and to have them active in the same phase of the cell cycle as in which the HR takes place. ZNF do not have a perfect specificity: if the specificity is too low and the ZNF nuclease introduces too many breaks, it becomes toxic. ZNF nucleases induce DSB and are repaired every time the cell divides. This makes them a bit out of phase with the HR since HR is another event and transcription dependent, happening in S-phase. TAL Nuclease is a bit more specific being fusion proteins consisting of DNA binding and DNA cleavage 'lego' buildingstones. This was a very interesting session with good overviews and outlooks and new challenging ideas. Since the approach of Mario Hermann was a good example of this, he was the second speaker receiving the poster award. To use the protein scaffolds most efficiently we must understand the mechanisms behind their action but, above all, their specificity and activity in phases of the cell cycle and during development. This would be a very nice and rewarding topic for another symposium or workshop.

Session 5 was an update about the use of miRNAs. miRNAs are also used for reverse genetic studies and emerge to become a viable alternative to knock-out gene activities by knocking activities down. Kai Schönig showed the applicability in transgenic rats and Jost Seibler showed a general applicable system for temporal control of ubiquitous miRNA expression in mice.

Session 6 was about applications and models. Werner Müller used oligo nucleotide-mediated gene modification to study the loss of function of defensin genes, Andrii Domansky applied BAC based transgenesis together with tamoxifen inducible Cre recombination in the study of pTEN ablation in neurons which protected in Parkinson's disease models and Erika Cantelli showed the use of Positron Emission Tomography to evaluate tumor metabolism and response to therapy as an imaging technique.

Last but not least session 7 was devoted to the resources. Birling presented Cre transgenic mouse lines expected to express the tamoxifen inducible CreERT2 recombinase in different target tissues and cells. They are made available to the research community by the Institut Clinique de la Souris (ICS) and for details you can visit http://www.ics-mci.fr/mousecre/ or www.creline.org. Johannes Schenkel showed how they manage the major data stream of genetically modified mice at the German Cancer Research Center in Heidelberg.

The workshop was again concluded with a forum discussion, the favourite part of the workshop. Hein te Riele moderated the discussion in his provocative but very elegant way and focussed on the topics nucleases, 2i medium and F0 mice. On the first topic of nucleases very positive reactions came from the use of TALEN's, they seem to be more specific, more custom made, more accessible with prices in the range of 800-5000 euros. With respect to HR and injection of nucleases in the fertilised oocyte the suggestion was done to try the application in un-fertilised oocytes since the process of HR has higher activity than the end ligation, knowledge that comes from Xenopus. The 2i medium was compared to the growth on feeders with LIF; when growing on 2i there is the behaviour of floating, not a real problem but some like their cells sticking, they should move back to feeders. Stability on the long run in 2i is not known!! Again a reason to go back to feeders. 2i medium just 2-3 days before injection gives better chimeras. 2i Medium is needed for rederivation of new lines, but not for HR. The generation of F0 mice as model was discussed. There was agreement on the fact that you always need to generate two cohorts: the experimental cohort and a control cohort, WT mice are not the proper control. F0 mice from ESC seem to be more reliable than iPSC since they are raised against the selective power. iPSC are selected for fast growing cells and certainly in cancer research that is not what you want.

Again the 6th IMM workshop was an interesting one with ample opportunity to talk to colleagues and discuss positive and negative results and to visit the booths with commercial partners that made the financial realisation of the workshop possible against a fair fee. We like to thank all the participants for being there and all our commercial partners the ISTT, Tecnilab-BMI, Cryosolutions/Planer, Stemcell Sciences, Axon Medchem, Merck Millipore and Parallabs for their support.

We hope to see you all again on the 7th edition of the IMM in 2013!!