CT1: CRISPR gene editing. Proceed with caution

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Recent clinical trials using CRISPR/Cas9 gene edited hematopoietic stem cells have yielded remarkable results in patients with sickle cell disease and beta-thalassemia. However, safety concerns regarding the therapeutic use of CRISPR remain which need to be addressed for the full potential of gene editing to be realised in a clinical setting. The ability to introduce unwanted mutations in the genome could negate the therapeutic effect of CRISPR/Cas9 and potentially lead to the development of cancer. To mitigate CRISPR/Cas9 off-target effects we developed COSMID[1] a bioinformatic tool to pre-screen gRNAs for off-target potential and demonstrated that a high-fidelity variant of Cas9 significantly reduced off-target mutations[2]. We found evidence of clonal hematopoiesis in a pre-clinical xenograft model of gene editing hematopoietic stem cells which prompted us to develop TRACE-seq a barcoding strategy to track clonal expansion in gene edited cells *in vivo*[3]. TRACE-seq identified a high degree of clonal expansion in gene edited stem cells which may have implications for long term screening of patients who receive gene edited cells. Pre-existing immunity towards Cas9 in humans has been reported by several groups. To understand the impact that pre-existing immunity could have on *in vivo* gene editing we used a mouse model to demonstrate that while gene editing was successful in mice immunized against Cas9 protein, all gene edited cells were eventually removed by the host immune system[4]. Our data suggests that the use of CRISPR *in vivo* should aim for short term and tightly regulated expression of Cas9 protein and potentially use supporting short immune immunosuppressive therapy.

1. Cradick et al. (2014). *PMCID: PMC4272406*
2. Park et al. (2019). *PMCID: PMC6735704*
3. Sharma et al. (2021). *PMCID: PMC7817666*
4. Li et al. (2020). *PMCID: PMC7264438*

CT2: Gene-editing for the clinic

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Efficient and safe gene-editing has the potential to treat many of the 5,000 monogenetic diseases that affect 250 million people globally. Conditions that affect either skin or blood cells are attractive targets for gene-editing applications due to their ease of access.
In our skin research we are targeting a severe, monogenetic fragile skin condition called epidermolysis bullosa (EB). To date there is no treatment for this condition. Our ultimate aim is to engineer gene-edited, patient-specific skin *in vitro* which can be used to permanently cover the chronic wounds of people with EB. We have optimised CRISPR/Cas9 editing methods in healthy and patient-derived primary keratinocytes and fibroblasts (the two main cell types present in the skin). We can achieve >95% non-homologous end joining (NHEJ)-mediated repair efficiencies in both cell types. Preliminary data suggest that these rates will allow for a successful exon-skipping approach to remove defective exons and thus repair *collagen 7* in patient-derived cells. Other EB-causing mutations require a homology directed repair (HDR) approach to make specific edits in the gene of interest. To date we have achieved a clinically useful rate of > 65% targeted edits in patient-derived keratinocytes. Off target and functional analyses are currently in progress.
In our T cell research we are dissecting immune checkpoint inhibitor biology by editing primary human T cells and melanoma cell lines to fine-tune some of the key molecules involved in these pathways (specifically PD-1 and PD-L1 respectively[1]). We can achieve high knock-out rates in both cell types (>95%) and targeted knock-in rates (HDR) of >65% in T cells and >98% in melanoma cells. The functional consequences of modulating these pathways are currently being analysed. Results from this research will have implications for improving checkpoint inhibitor therapies and to engineering an optimal cancer-killing T cell for use in adoptive immunotherapies.

1. Michaels Y, Barnkob B, Barbosa H, Baeumler T, Thompson M, Andre V, Colin-York H, Fritzsche M, Gileadi U, Sheppard H, Knapp D, Milne T, Cerundolo V, Fulga T *Precise tuning of gene expression levels in mammalian cells.* Nat Commun, 2019. 10(1): p. 818.

CT3: GCVL – A tool to disentangle different biological effects in pooled CRISPR/Cas9 knockout screens

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Pooled CRISPR/Cas9 knockout screens are a powerful functional genomics technique that involves investigating gene function in a systematic and high-throughput fashion. This allows us to interrogate the function of a large number of genes – potentially the entire genome – in a single experiment. Our laboratory has implemented a CRISPR/Cas9-knockout functional genomics pipeline using the whole genome GeCKOv2 and Brunello libraries, as well as smaller scale focused libraries. Using this pipeline, we have recently conducted functional genomics screens using the Brunello library to identify genes that are essential in mediating the tolerance of head and neck squamous cell carcinoma (HNSCC) cells to hostile stressors in the tumour microenvironment (TME), notably low oxygen (hypoxia), glucose depletion and an acidic extracellular environment. Analysis of this data set, however, was complicated by differential cell growth between our control and treatment conditions, which cause bias in the detection of knockouts affected by the treatment alone (e.g. hypoxia).

We have developed a novel data analysis method, called growth-corrected voom limma (GCVL) that allows us to deconvolute the treatment effect from the effect of cell growth. GCVL makes use of the expected biological behaviour of gene knockouts, as well as the highly parallel nature of our screen data, to robustly estimate growth-related covariates from the data itself. These covariates are then incorporated into a statistical model to correct for variable growth between samples. Using GCVL, we were able to effectively disentangle the growth-related effects from the treatment effects in our TME screens to greatly improve hit calling for these screens. We are currently validating these hits using chemical inhibitors and single-gene knockout experiments. In conclusion, GCVL is a new tool for analyses of functional genomics data that can be used to correct for cell growth-related effects and improve detection of treatment effects in typical control-treatment experimental designs.

CT4: Lessons from genetic screens and single-gene knockouts with the CRISPR/Cas9 system

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Pooled CRISPR/Cas9 knockout screens allow the parallel evaluation of phenotypes of individual gene knockouts. The initial cell population consists of cells each bearing inactive copies of a single gene but collectively representing knockout of all genes of interest. Such library can be used to identify growth essential genes, synthetic lethal interactions, or genes that confer sensitivity or tolerance to external stimuli, i.e. drugs or physiological stress. Follow-up phenotype studies using isogenic single gene knockout cell lines enable validation of the screen findings and provide opportunity for identification of the underlying mechanism.

In our laboratory, we utilise CRISPR/Cas9 knockout screening technology and isogenic models to identify novel therapeutic targets for cancer and predictive biomarkers for anticancer therapies. In developing our technological pipeline, we have taken a series of steps to optimise CRISPR/Cas9 screen performance and single gene knockout specificity. This includes the use of CRISPR technology variants to select Cas9 expression mode (constitutive or inducible), method of knockout generation (small indel or large deletion), and guide RNA delivery (plasmid or ribonucleoprotein complex). I will present how we have applied these technological advancements using specific examples from our screens for genetic dependencies of cancer cell sensitivity to antibody-drug conjugates, radiation, and DNA-targeting agents.

CT5: Investigating DNA methylation as a mechanism for paradoxical gene activation using targeted epigenetic editing

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Advanced melanoma is well known for its propensity to metastasize, with 80% of patients presenting brain metastasis at the autopsy. As such, it is imperative to investigate the metastatic process and to develop new treatment options. DNA hypermethylation at gene promoters has been widely established as a strong down-regulator of gene expression in many diseases, particularly in cancer. However, recent studies from our group and several others have demonstrated the opposite phenomenon, where high gene expression occurs in the presence of high promoter methylation, challenging the dogma of promoter methylation being only a silencing mechanism and raising the possibility that for a group of genes, promoter hypermethylation may activate gene expression.

Established methylation manipulation methods commonly involve the use of demethylating agents such, as 5-azacytidine, which act globally, altering methylation levels in a replication dependant manner. Thus, to precisely investigate the activating role of promoter hypermethylation, we employed a novel methylation-editing approach using CRISPR-based editing technology. We targeted a 58 bp region of the *EBF3 (early B cell factor 3)* gene promoter, which we previously identified as a putative 'epigenetic driver' of melanoma metastasis. Using lipofectamine based transfection method, our results showed both successful methylation and demethylation of this target locus across multiple cell lines.

We now strive to correlate these locus-specific changes in methylation with gene expression, and subsequently, corresponding changes in the chromatin-associated protein binding using CUT and RUN assay. Consequently, we aim to determine whether decrease in promoter DNA methylation result in the repression of gene expression in this context. Our study challenges the long-standing notion of methylation as an exclusively silencing mechanism and aims to establish a new paradigm. The findings from this study may also help unveil epigenetic drivers of malignancy and metastasis.

CT6: Dissecting the PD-1/PD-L1 axis in melanoma using a microRNA-based fine-tuning approach

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Immune checkpoint inhibitors (ICIs) have revolutionised the treatment of certain cancers such as melanoma. ICIs unshackle our immune cells to kill cancer. One type of ICI works by blocking the interaction of the PD-1 receptor on immune T cells with its ligand PD-L1 on the cancer cell. ICIs targeting the PD-1/PD-L1 axis are an effective treatment in approximately 30% of melanoma patients[1], however the underlying biology remains poorly understood. PD-L1 expression alone is a poor biomarker for patient response to ICIs. 30% of PD-L1+ patients fail to respond[2], while up to 15% of PD-L1- patients do respond to ICI therapy[3]. Additionally, the role of a second PD-1 ligand (PD-L2) in the response to treatment is poorly characterised. Therefore, a fuller understanding of the PD-1 checkpoint pathway is needed to better predict which patients will benefit from ICI treatment.

Here we aim to fine-tune endogenous PD-1 and PD-L1/PD-L2 expression in T cells and melanoma respectively, at physiologically relevant levels. We hypothesise that there are optimal expression levels of PD-1/PD-L1 axis proteins which tip the balance towards either immune escape or cancer cell killing. MicroRNA based fine-tuning of endogenous gene expression is achieved through CRISPR/Cas9-mediated insertion of synthetic microRNA response elements into the 3'UTR of target genes[4].

To-date we have demonstrated up to 98% targeted insertion of a miRNA response element into the target genes PD-1 and PD-L1 in CD8+ T cells and a melanoma cell line, respectively. Flow cytometric analysis of PD-L1-edited cells showed a concurrent decrease in cell surface protein expression indicative of fine-tuned gene expression. Additionally, we have generated highly efficient (>90%) PD-L1 and PD-L2 knockout melanoma cells. Experiments are underway to assess the functional impact of melanoma PD-L1/PD-L2 modulation on melanoma-specific T cells. We expect our findings will have implications for many cancer types.

1. Gellrich, F.F., et al., *Anti-PD-1 and Novel Combinations in the Treatment of Melanoma-An Update.* J Clin Med, 2020. **9**(1).
2. Morrison, C., et al., *Predicting response to checkpoint inhibitors in melanoma beyond PD-L1 and mutational burden.* J Immunother Cancer, 2018. **6**(1): p. 32.
3. Sunshine, J. and J.M. Taube, *PD-1/PD-L1 inhibitors.* Curr Opin Pharmacol, 2015. **23**: p. 32-8.
4. Michaels, Y.S., et al., *Precise tuning of gene expression levels in mammalian cells.* Nat Commun, 2019. **10**(1): p. 818.

CT7: Editing DNA methylation in cancer cells

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DNA methylation is a key epigenetic modification implicated in the pathogenesis of numerous human diseases, including cancer development and metastasis. Gene promoter methylation changes are widely associated with transcriptional deregulation and disease progression. Longstanding techniques for the investigation of DNA methylation in disease etiology tend to manipulate the methylome on a global scale and are severely limited with regards to locus specificity. Therefore, it has been difficult to establish causal relationships between aberrant methylation changes and disease. The advent of CRISPR-based technologies, however, has provided a powerful tool for locus-specific manipulation of the epigenome. Here, we describe a simple protocol for the design and application of a CRISPRdCas9-based tool for editing DNA methylation at a target locus in human melanoma cell lines, alongside protocols for downstream techniques used to evaluate subsequent methylation and gene expression changes in methylation-edited cells.

CT8: 'Squeezing DNA into the active site' – How ABEs edit the genome

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CRISPR-Cas9 base editors are promising tools for highly site specific editing of DNA. Adenine base editors (ABEs) are a fusion of a laboratory evolved bacterial tRNA adenosine deaminase (TadA) and the RNA-guided Cas9. In its native context, TadA specifically loads a tRNA anticodon loop into its active site to catalyse the deamination of adenosine to inosine. In the context of ABEs, the TadA domain was selected by directed evolution to catalyse the deamination of deoxyadenosine to deoxyinosine, a chemical conversion that can be read by genome maintenance machinery as a deoxyguanosine to drive a desirable repair outcome.

To better understand the molecular mechanism of these precision genome editors and enable further development, we determined the first structure of an ABE[1]. By incorporating 8-azanebularine into the DNA substrate, we trapped and visualised the DNA bound and substrate loaded state of ABE8e by cryo-electron microscopy. The structure revealed how the TadA domain captures single-stranded DNA presented by Cas9 to load the substrate into the active site in a constrained tRNA-like conformation. The structure also reveals the determinants for ABE editing windows and underscores that base editor precision likely exploits the kinetics of the deaminase domain. These results not only explain ABE-mediated base-editing outcomes but inform the future design of base editors

1. Lapinaite, A and Knott GJ *et al*., (2020) *DNA capture by a CRISPR-Cas9 guided adenine base editor*. Science. 369:566-571.

CT9: Developing a CRISPR-Cas9 system in *Penicillium paxilli* for secondary metabolite production

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Over millions of years of evolution, fungi have built a large array of complex secondary metabolites with wide-ranging bioactive properties. Our group uses synthetic biology approaches to synthesise and characterise these secondary metabolites in order to determine their bioactive potential. We are currently interested the nodulisporic acids, which are indole diterpene secondary metabolites with potent anti-insectan activity and low mammalian toxicity. We have recently developed a CRISPR-Cas9 system in our fungal host, *Penicillium paxilli*, to integrate components of the nodulisporic acid biosynthetic pathway into a desired location within the *P. paxilli* genome. Our CRISPR-Cas9 approach involves the use of a ribonucleoprotein complex of Cas9 protein and guide RNAs, and a plasmid template designed to facilitate homologous recombination at the desired genomic location. Using this CRISPR-Cas9 approach, we improved our rate of targeted integration of the nodulisporic acid biosynthetic genes from below 10% to over 90%, while also greatly increasing our overall transformation efficiency. We have successfully used this approach to integrate up to nine biosynthetic genes in our desired location. We are currently working to expand our CRISPR-Cas toolkit to include applications such as CRISPR activation. By developing a CRISPR-Cas toolkit, we aim to create a tuneable system where we can integrate and control secondary metabolite biosynthesis allowing us to generate commercially viable yields of target compounds.

CT10: Inverse regulation of CRISPR-Cas and surface-based immunity discriminates plasmids versus phages

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Bacteria harbor multiple lines of defense against bacteriophages and mobile genetic elements. CRISPR-Cas systems represent the only known prokaryotic adaptive immune response, during which 'memories' of past infections facilitate future immunity against the same foreign invader. While beneficial for defense, CRISPR-Cas systems can impose fitness costs on the host[1], including self-targeting[2,3], as well as representing a potential barrier to beneficial horizontal gene transfer. Identifying regulatory networks controlling CRISPR-Cas immunity is crucial to understanding when adaptive defense is favored or dispensable, in order for cells to balance fitness costs with defense. To systematically uncover regulators of CRISPR-Cas immunity, we first had to overcome a lack of suitable high-throughput genomic tools. We developed the SorTn-seq method[4], which employs fluorescence activated cell sorting to enrich mutants with altered fluorescent reporter activity from within a saturated transposon mutant pool. Sorted cells are then deep sequenced to locate transposon insertion sites to identify putative regulators of the gene of interest. We applied SorTn-seq to assess *csm* (type III-A CRISPR-Cas) gene expression in ~300,000 unique mutants of the enterobacterium *Serratia* sp. ATCC 39006[5]. We identified several genes implicated in regulation of the type III-A CRISPR-Cas system, including those involved in resource utilization, motility, and stress response. Activation of the Rcs outer-membrane stress response system repressed adaptive immunity by three distinct CRISPR-Cas systems, while coordinately promoting cell surface-based immunity against diverse phages. Our results suggest that cell stress can differentially control bacterial immune strategies, which has important consequences for horizontal gene transfer.

1. Vale, P. F. *et al.* (2015). *Costs of CRISPR-Cas-mediated resistance in Streptococcus thermophilus*. Proc. R. Soc. B. 282, 20151270.

2. Stern, A., Keren, L., Wurtzel, O., Amitai, G. & Sorek, R. (2010). *Self-targeting by CRISPR: gene regulation or autoimmunity?* Trends Genet. 26, 335-340.

3. Vercoe, R. B. et al. (2013). *Cytotoxic chromosomal targeting by CRISPR/Cas systems can reshape bacterial genomes and expel or remodel pathogenicity islands*. PLoS Genet. 9, e1003454.

4. Smith, L. M., Jackson, S.A., Gardner, P.P., Fineran, P.C. (2021). *SorTn-seq: a high-throughput functional genomics approach to discover regulators of bacterial gene expression*. Nat. Protoc. In press.

5. Smith, L.M., Jackson, S.A., Malone, L.M., Ussher, J.E., Gardner, P.P., Fineran, P.C. (2021). *The Rcs stress response inversely controls surface and CRISPR–Cas adaptive immunity to discriminate plasmids and phages*. Nat. Microbiol. 6, 162-172.

CT11: Type III CRISPR-Cas enables resistance against nucleus-forming jumbo phages

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Many prokaryotes encode CRISPR-Cas systems as immune protection against bacteriophages. Importantly, jumbo phages (>200 kb genome) are able to evade DNA-targeting CRISPR-Cas immunity by physically protecting their DNA inside a viral nucleus-like structure during infection. However, phage mRNA remains vulnerable to RNA-targeting CRISPR-Cas systems (type III) when exiting the nucleus for translation. It is unknown as to how the type III systems provides protection from jumbo phages since the Cas interference complex is unable to access the phage DNA, and the typical type III model eventually requires access to, and destruction of, the phage genome by Cas10. Here we present evidence of our current model of how the type III system can provide jumbo phage resistance in *Serratia*. Our results highlight the diversity of strategies used in CRISPR systems to provide immunity. The ability of jumbo phages to escape from DNA-targeting innate and adaptive immune systems can be exploited as antimicrobials in phage therapy.

CT12: Insights into anti-CRISPR control by the autoregulator Aca2

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Bacteria are under constant threat of invasion by bacteriophages (phages) and mobile genetic elements. Bacterial CRISPR–Cas systems are adaptive immune defences targeting such invaders. In response, Phages have evolved anti-CRISPR (Acr) proteins which can inhibit different types of CRISPR–Cas systems in various ways. Acrs are therefore highly diverse but are frequently encoded in an operon with a more conserved anti-CRISPR-associated (*aca*) gene. All known Aca protein families are predicted to contain a helix–turn–helix (HTH) domain, which led to the proposal that they might serve as regulators of *acr* expression. However, this hypothesis has long remained untested.

We investigated whether Aca proteins regulate their *acr–aca* operons by examining the *acrIF8–aca2* locus of phage ZF40, which infects the plant pathogen *Pectobacterium carotovorum*. The *acr* promoter contains two conserved inverted repeat (IR) pairs which we hypothesized might act as binding sites for Aca2. Using reporter assays, we showed that Aca2 is a repressor of the *acrIF8–aca2* promoter and that its HTH domain is essential for repression. These experiments further revealed that one of the IRs is bound with high affinity and mediates repression both upon *aca2* overexpression and upon low-level expression by a ZF40 prophage. In contrast, the other IR appears to mediate repression only at high Aca2 concentrations.

Based on our previous findings, here we present our current understanding of Aca2-mediated anti-CRISPR regulation. These results shed light on the complex regulation of phage counter-defence against their hosts' CRISPR–Cas systems and might have implications for the deployment of Acr proteins in CRISPR-based technologies.

CT13: What are they do*ING* in flowering time and development? Gene editing of *INHIBITOR OF GROWTH* genes in the model legume *Medicago*

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Abstract

Legumes are important forages, oil crops and food, but are generally an under-optimized agricultural family, with the exception of the major economic crop soya bean. One important trait key to improving productivity and yield is optimised flowering time, as a prelude to successful sexual reproduction and the production of seeds and grains. Here we present CRISPR-Cas9 gene editing of two *INHIBITOR OF GROWTH* (*ING*) genes, members of a group of conserved genes found across all eukaryotes and usually found as two genes in plants. There are no reports of plant *INGs* analysed by mutation, thus we gene-edited *ING1* and *ING2* in the model legume *Medicago* to obtain knock out alleles to determine their function *in vivo*. Gene editing also provided the opportunity to probe the importance of different parts of the ING proteins (an N-terminal coiled-coil ING domain and a C-terminal Plant Homeodomain (PHD) finger). A multiplexed array of 6 or 7 guides was expressed from a Medicago U6 promoter and introduced into plant leaf cells using *Agrobacterium*, from which transformed plants were regenerated, genotyped and self-fertilised to generate homozygous mutagenised plants. Loss of *MtING2* has significant and varied effects on plant development including causing late flowering time, compound leaf patterning, trichome development and plant architecture. RNA Seq and ChIP-Seq indicate global changes to gene expression, including reduction in expression of a key promoter of flowering *MtFTa1*, and the location of H3K4me3 peaks in the *Mting2* mutant. Interestingly, the predicted loss of some highly conserved amino acids in the PHD finger does not seem to disrupt ING2 function in some alleles. In contrast, the role of *MtING1* is less clear, although it may still have a significant effect on plant development and this is being tested in double *Mting* mutants. Although well studied in humans and yeast, much remains to be discovered about *ING* genes in plants. Thus this work on legume *INGs* contributes pioneering fundamental knowledge on the role of INGs in plants development.

CT14: Fine Tuning CRISPR/Cas9 for Generating Genetically Altered Rodents

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Creating genetically altered (GA) rodents to mimic human ailments is an invaluable tool in biomedical research. We have come a long way since the days of random targeting to targeted genome editing. Engineering advancements have facilitated genome editing tools such as meganucleases, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs) and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) (CRISPR/Cas9). Here, we compared several methods to produce GA rodents from embryo microinjection to *in vivo* electroporation to i-GONAD (improved genome-editing via oviductal nucleic acids delivery). We also tested all-in-one plasmid, Cas9-mRNA vs protein, and crRNA+tracrRNA vs sgRNA in different combinations. We successfully produced genetically altered mice on the C57BL/6J background, and rats on the Wistar and Sprague Dawley backgrounds using one or more of these combinations [one knockout mouse (8 founders), 3 knockout rats (totalling 17 founders), 3 knockin mice (totalling 14 founders), and 1 knockin rat (8 founders)]. From our trials so far, we have made the following observations: 1. The use of ribonucleoprotein (sgRNA+Cas9 or crRNA+tracrRNA+Cas9) complexes were better than using Cas9 mRNA. 2. *In vivo* electroporation of embryos has the potential to replace microinjection as a method of choice for reagent delivery to produce knockouts and small knockins. 3. i-GONAD has the potential to significantly reduce the number of animals required to generate the knockouts in mice, but microinjection and *in vitro* electroporation are still the best options for small knockins. 4. Single-strand DNA (ssDNA) works well as a donor for small inserts. 5. Microinjection is still the go-to method for inserting transgenes or large constructs. From these observations, we conclude there is a need to pick the method based on the type and size of targeted editing.

CT15: Fetal kidney complementation in gene edited, immune-compatible sheep

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Farm animals have been proposed as hosts to grow human organs for xenotransplantation. This concept involves genetically disrupting target organ development in the livestock host, followed by complementation with human cells that populate the vacant niche and generate the transplantable organ. In mice, spalt-like transcription factor 1 (*Sall1*) functions as a master regulator for kidney development[1]. To generate anephric, immune-compatible hosts for organ complementation, we targeted *SALL1*, as well as two major xenoantigens involved in hyperacute immune reaction: galactose-α(1,3)-galactose (α-Gal) and N-glycolylneuraminic acid (Neu5Gc). We chose sheep as the host species because they have a similar kidney anatomy to humans but are perceived as culturally more acceptable organ donors than pigs.

Using Cas9 editing, we simultaneously disrupted *SALL1* and the genes underlying α-Gal and Neu5Gc formation, α(1,3)galactosyl transferase (*GGTA*) and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (*CMAH*), respectively. Ovine fetal fibroblasts (OFFs) were transfected with plasmids containing CRISPR guide RNA sequences for each different target gene. Double (CMAH/GGTA) and triple (CMAH/GGTA/SALL1) knockout clonal strains were sequence-verified before use in somatic cell transfer cloning to produce edited fetuses and animals. Five female CMAH-/- GGTA-/- double knockout ewes were produced and confirmed negative for Neu5Gc and α-Gal. *SALL1* targeting after the fourth zinc finger domain produced mild to severe kidney, while removing all zinc finger domains resulted in a single fetus with kidney agenesis.

For kidney complementation, rescue of the anephric phenotype was examined in intraspecific fetal chimaeras between triple knockout hosts and mCherry fluorescent double knockout donors. High contribution of mCherry donor cells rescued kidney development, while low level mCherry contribution produced fetuses with kidney hypoplasia similar to the original SALL1 knockout fetus. Overall, this study establishes sheep as an alternative model for xenotransplantation research.

CT16: Exploring the role for the gout susceptibility gene *ABCG2* during acute gouty inflammation

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Gout represents the most common inflammatory arthritis in New Zealand, with Māori and Pacific peoples showing some of the highest prevalence rates worldwide. In gout, hyperuricemia can lead to monosodium urate (MSU) crystal deposition in peripheral joints, which activates tissue-resident macrophages that drive neutrophil recruitment and episodes of painful acute inflammation. Hyperuricemia can have a multitude of causes, one of which is a genetic predisposition, which favours the early development of gout.
Single nucleotide polymorphisms (SNPs) in the gout susceptibility gene *ATP-binding cassette subfamily G member 2* (*ABCG2*) can contribute to the development of hyperuricemia due to decreased urate transporter function. Notably, even after hyperuricemia has developed in gout patients, carriers of specific SNPs in the *ABCG2* gene show a higher prevalence of developing gout flares, suggesting an additional role for this transporter in the context of inflammation.
Taking advantage of the genetic tractability and optical transparency of zebrafish larvae, we set out to investigate the role of the *ABCG2* homologue, *abcg2a,* in our unique model of acute gouty arthritis. We pursued two CRISPR-Cas9 strategies to explore Abcg2a function. First, we created a null allele using non-homologous end-joining and more recently established a mutation that functionally mimics the most prevalent *ABCG2* SNP in humans using homology-directed repair and which is predicted to encode a loss of function variant. This strategy enabled us to quickly discover phenotypes in larvae homozygous for the null allele, including a prolonged period of neutrophilic inflammation (the hallmark of acute gouty inflammation) in response to MSU crystals. Our plan is to investigate these phenotypes in the SNP mutant and the underlying molecular mechanism. These mutant lines will provide a valuable resource to understand the role of ABCG2 in immunity and how it contributes to acute gouty inflammation.

C1T7: Progress toward generating a Sheep (*Ovis aries*) model of Alzheimer's Disease

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Animal models of Alzheimer's Disease (AD) have so far failed to provide the much sought after cure or prevention method. One of the biggest problems in the field has been translatability between the rodent models and clinical trials. A number of different mutations have to be overexpressed in rodent models in order to generate a complete AD-like phenotype. Some of the mutations are not found in human AD, which leads to questions about the validity of rodent models. There has been a steady increase in calls for more valid models of AD over the last decade. This project is to create a sheep (*Ovis aries*) model of Alzheimer's Disease. The aim is to introduce the E280A mutation into the *PSEN1* gene of sheep using CRISPR-Cas9 technology. Sheep have much larger brains than rodents and our previous research has shown that plaques and tangles, which are the hallmarks of AD, develop naturally in these animals with age. Sheep have a longer lifespan, and as the gene will be expressed under its natural promoter, it will most likely reflect the normal biological function of this allele in human patients. This will provide a more valid and robust model for therapeutic testing. In the process of generating a sheep AD model, we inadvertently created several *PSEN1* knock out sheep. These will be useful for research into the function of *PSEN1* and for crossing with our E280A knock in model. We have also trialled three different CRISPR-Cas9 guide systems and two different preimplantation testing methods. 17 embryos heterozygous for the E280A substitution were implanted last year and 11 lambs have been born. We are in the process of characterizing them and the results are promising so far.

CT18: NT2/D1-cas9: a novel in vitro model for male human disorders of sex development

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Differences of Sex Development (DSD) are a spectrum of conditions in which gonadal, chromosomal or anatomical sex is atypical. The underlying molecular basis remains unknown in many forms of DSD, due in part to the lack of manipulable models that recapitulate human sex determination. Sex is determined by the onset of SRY expression around week 6 in the human XY embryo. SRY mutations account for 15% of 46,XY DSDs; autosomal genes such as SOX9 also contribute. Mouse models have been useful in studying the function of critical sex determination genes using knockout/transgenic approaches, yet there are limitations when exclusively relying on these models. Functional redundancy, gene dosage or genetic buffering (alternative pathways existing for the same functional outcome) often result in no phenotypic consequence[1]. Furthermore, differences of gene expression thresholds and genetic robustness between humans and mice are becoming apparent[2]. An in vitro model that can be used to model Sertoli cell function in the testis is NT2/D1, a human pluripotent clonal cell line derived from a testicular tumour[3]. Expressing the testisdetermining genes SRY, SOX9, SF-1, DHH and FGF9, with an absence of ovary-specific FOXL2 and WNT4, NT2/D1 cells can be used in their undifferentiated state as a model for human Sertoli cell function. We have established an NT2/D1-cas9 cell line and characterised these via a suite of cell phenotyping assays including xCELLigence® RTCA and HoloMonitor® live cell imaging. This model can be used in conjunction with siRNA knockdown investigations of the multiple SOX9 target genes, to assess their individual contributions to cell adhesion, proliferation and polarity – all important behaviours of Sertoli cells to direct testicular architecture in the developing male gonad. Cas9 engineered deletion and missense mutations from DSD patients will help to pinpoint the processes that differ in cells in XYfemale versus typical XY-males.

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CT19: Elevated endoplasmic reticulum calcium flux plays a vital role in dual-guide CRISPR/Cas9 generated type I calreticulin mutant cells

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Calreticulin (CALR) is a multifunctional soluble protein that regulates calcium homeostasis and protein folding in the endoplasmic reticulum (ER). Mutations in exon 9 of the *Calr* gene are the second most common genetic cause of myeloproliferative neoplasms (MPNs), with a characteristic increase of megakaryocytes (platelet precursors). Several studies demonstrate that CALR mutated megakaryocyte proliferation involves a cytokine-independent constitutive activation of JAK/STAT signaling due to the binding of mutant CALR to the thrombopoietin receptor. However, the impact of Ca^{2+} alterations in CALR mutations is unclear. To examine this, we employed a dual-guide CRISPR/Cas9 approach to generate the common type I CALR mutations (52-bp deletion) in human myelogenous K-562 *cells*.

Dual-guide CRISPR/Cas9 gene editing enables us to obtain single-cell clones with complete or partial type I CALR mutations, and the clonal validation performed by mass spectrometry and western blotting confirmed the secretion of mutant CALR. Interestingly, fluorescent-based calcium measurements with ER Ca^{2+} indicator Mag-fluo-4 AM showed that CALR mutant cells have higher intraluminal ER Ca^{2+} than WT K-562 cells. Moreover, the cytoplasmic calcium indicator Fura 2 AM displayed an elevated store depletion of Ca^{2+} upon ionomycin in mutant cells. We also found that increased ER calcium was not due to an altered ER capacity in the clones. Notably, type I CALR mutant cells gave rise to more $CD61^{+}CD41a^{+}$ megakaryocytes than WT cells upon PMA treatment. Most importantly, we discovered that type I CALR mutant cells had considerably higher basal phosphorylation of ERK1/2, which increased further upon PMA-stimulated maturation.

Overall, our results demonstrate that type I CALR mutant K-562 cells have elevated intraluminal ER Ca^{2+} flows and baseline activation of the mitogen-activated protein kinase (MAPK) pathway that causes downstream phosphorylation of ERK1/2. This finding suggests a possible involvement of calcium-activated signaling events in CALR mutant-driven megakaryocyte proliferation. Further studies are warranted to unravel the underlying mechanism of how elevated ER Ca^{2+} activates MAPK signaling to favor megakaryocyte proliferation in type I CALR mutant MPNs.

CT20: Successful modelling of a single nucleotide polymorphism in cells and mice using CRISPR-Cas9 and CRISPR-Cas12 methodologies

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The single nucleotide polymorphism (SNP) rs1800795 resides within the interleukin-6 (IL-6) gene promoter and alters *IL6* transcription[1]. The SNP has reported associations for multiple pathologies, including infections, inflammatory diseases, and the risk of various cancers. Importantly, the SNP roughly trisects the population and so has high value for clinical stratification of patients. Recently, we found that rs1800795 genotype also associates with disease recurrence in colorectal cancer (CRC) and so may have value as a prognostic biomarker in that malignancy as well as others. The advent of CRISPR-Cas9 based technologies allows SNPs to be rapidly modelled and directly studied. The *IL6* gene promoter is highly conserved between humans and mice with only minor differences in and around the rs1800795 SNP. But unfortunately, a difference did alter PAM site availability. We first established protocols based on Cas9 for cell lines of both species, but then moved to a Cas12 strategy to improve success rates for mouse cells. At the same time, we first attempted to generate genetically modified mice using Cas9, and then shifted later to Cas12 for that application as well. Success rates and additional unwanted modifications were mirrored between our cell line and animal work. We now have multiple useful modified cell lines and two different versions of rs1800795 mice. The animals display clear alterations in IL-6 regulation, and phenotypes that match the reported human associations for the SNP. The mice are powerful pre-clinical models for studying the prognostic and treatment response biomarker value of rs1800795, and reinforce the value of whole animal CRISPR approaches to studying SNP effects.

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Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
CT21	Investigating CRISPR/Cas13 as a novel tool for the knockdown of miRNA upregulated in diabetic heart disease (DHD)	Matthew Reily-Bell	Department of Physiology, Heart Otago, University of Otago School of Medical Sciences, Dunedin, NZ
CT22	Targeted epigenetic editing to elucidate the mechanism of MGMT gene regulation in melanoma	Nicolas Keestra	Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, NZ
CT23	Identification of genes involved in tolerance of tumour microenvironment stress using functional genomics	Hanting Yong	Auckland Cancer Society Research Centre, University of Auckland, Auckland, NZ
CT24	Identifying genetic dependencies in NRAS-mutant melanoma through whole-genome CRISPR/Cas9 screens in cell lines established from New Zealand melanoma patients	Andrea Gu	Auckland Cancer Society Research Centre, School of Medical Sciences, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ
CT25	*Ex vivo* gene therapy for epidermolysis bullosa; severe skin fragility disorders	John Hunt	Department of Biological Science, The University of Auckland, Auckland 1050, NZ
CT26	Disrupting the oligomeric structure of peroxiredoxins in cancer cells using CRISPR	Paul Pace	Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ
CT27	Exploring Gene Editing of Aryl Hydrocarbon Receptor Gene in Primary Wild Type and Leukaemia Bone Marrow Cells Using HDR Pathway of CRISPR-Cas System	Olena Oryshchuk	Leukaemia & Blood Cancer Research Unit, Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, NZ
CT28	Towards the development of ex vivo gene therapy for the severe group of fragile skin disorders epidermolysis	Alex Du Rand	Department of Biological Science, The University of Auckland,

	bullosa		Auckland, NZ
CT29	Identification of CRISPR-Cas and novel phage defence systems to expand our molecular toolkit	Leighton Payne	Department of Microbiology and Immunology, University of Otago, NZ

CT21: Investigating CRISPR/Cas13 as a novel tool for the knockdown of miRNA upregulated in diabetic heart disease (DHD)

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Diabetes Mellitus, an ever-growing epidemic, kills millions every year with ~50% of deaths attributable to DHD[1, 2]. MicroRNAs (miRNA) implicated in the development of DHD are being explored as potential biomarkers and therapeutic targets/agents. Research has shown that restoring miRNA to physiological levels positively impacts cardiac health in mice. Despite this there are serious concerns with the specificity, toxicity and ethicality of current technologies [3-5]. A new CRISPR approach, CRISPR/Cas13, bypasses ethically contentious genome modifications by targeted modification of RNA with greater specificity than both siRNA and antisense oligonucleotides. Currently, CRISPR/Cas13 has been used effectively to knockdown both mRNA and lncRNA. Additionally, Cas13 was shown to bind to miRNA, and that binding was sufficient to activate nuclease activity. Despite this there are currently no reports of CRISPR/Cas13 being used for the targeted knockdown of miRNAs [6-10]. This project aims to determine the feasibility of CRISPR/Cas13 targeted miRNA knockdown. Cardiomyocyte-specific CRISPR/Cas13 expression plasmids have been designed to target miRNAs upregulated in the diabetic heart. The system will first be tested in cultured cardiomyocytes before being tested in diabetic mice. Plasmids will be packaged in AAV and/or nanoparticles for direct delivery, to the heart, via myocardial injection in diabetic mice. Cardiac performance will be monitored using echocardiography, and measurement of diagnostic markers in the blood. Rt-qPCR and RNA-Seq will be conducted, for multiple tissue types, to measure changes in RNA levels and search for off target effects. Target proteins levels will be measured by western blot. Effects on angiogenesis, myofibroblast differentiation and apoptosis will also be measured. Determining if CRISPR/Cas13 can mediate the knockdown of specific miRNA could provide the basis for a single dose treatment, that could improve cardiac health, over the lifetime of diabetic patients. This technology could also provide new therapeutic options for other diseases associated with overexpressed miRNA.

CT22: CRISPR Methylation-Editing Screen to Identify Drivers of Tumour Metastasis

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Although the overwhelming majority of cancer deaths are caused by tumour metastasis, the underlying molecular events that cause it have not yet been fully elucidated [1,2]. This limited understanding obscures our knowledge of the mechanisms driving metastatic progression and hinders the development of effective cancer treatments. DNA methylation is a stable and somatically heritable epigenetic modification that modifies gene expression and is commonly aberrant in cancer, especially in relation to metastasis [3]. Until recently it has not been possible to directly demonstrate that specific methylation changes alter metastatic potential. However, the development of precision editing tools now provides an opportunity to specifically edit epigenetic states (i.e. DNA methylation) of target genes and to exclusively investigate the effect of these changes on cancer cell function [4].

Our proposed research will further develop CRISPR/Cas technology to enable high-throughput interrogation and functional investigation of epigenetic drivers of metastasis. We aim to clearly demonstrate that an epigenetic mechanism drives tumour metastasis, with a focus on colorectal cancer (CRC). This will open new avenues for understanding metastasis biology, lead to better outcome prediction, and identify new targets to treat aggressive tumours in the future.

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CT23: Identification of genes involved in tolerance of tumour microenvironment stress using functional genomics

Yong, H.[1], Bhatta, S.[1], Singleton, D.C.[1,2], Lipert B. [1,2], Hunter, F.W.[1,2,3], Bohlander, S.K.[2,4], Print, C.G.[2,4], Wilson, W.R.[1,2], Jamieson, S.M.F.[1,2,5] and Lee, T.W.[1,2]

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The tumour microenvironment is often hypoxic and low in nutrients due to the chaotic and leaky nature of tumour vasculature. The altered metabolism adopted by tumour cells to proliferate in these conditions leads to the accumulation of acidic metabolites in the extracellular space, resulting in tumour acidosis. Adaptive mechanisms used by tumour cells to survive and thrive in the presence of these microenvironmental stressors are thus potential tumour-selective therapeutic targets. Further understanding of molecular players involved in tumour microenvironment stress tolerance will potentially provide novel drug targets and greater insight into tumour microenvironment biology.

To identify genes that confer tolerance to the physical tumour microenvironment stressors in an unbiased manner, we have performed whole-genome pooled CRISPR-Cas9 knockout screens in UT-SCC-54C, a head and neck squamous cell carcinoma cell line. UT-SCC-54C cells were first transduced with the whole-genome Brunello guide RNA (gRNA) library. The resultant knockout cell populations were then subjected to chronic hypoxia, glucose deprivation or acidosis, with screens for each stressor carried out on two independent transduction replicates. Sequencing of gRNAs and bioinformatics analyses of the gRNA counts have identified gene knockouts that are altered in frequency under the respective stressors. Significant survival advantage or disadvantage displayed by several knockouts in each screen suggest the involvement of pathways not known to contribute to tumorigenesis, or do so in unexpected ways. Potential gene candidates generated from the screens are currently undergoing validation.

CT24: Identifying genetic dependencies in *NRAS*-mutant melanoma through whole-genome CRISPR/Cas9 screens in cell lines established from New Zealand melanoma patients

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New Zealand has the highest susceptibility of malignant melanoma in the world. Mutations in *BRAF* and *NRAS* are found in approx. 50% and 20% of melanoma cases, respectively, are mutually exclusive and drive disease progression. Despite the success of targeted inhibitors of the RAS-RAF-MAPK signalling pathway for treatment of metastatic melanoma harbouring *BRAF* mutations, there is no effective treatment specifically for *NRAS*-mutant melanoma. Therefore, there is an unmet need for novel strategies for the therapy of *NRAS*-mutant melanoma. A current approach to identify novel drug targets and to overcome drug resistance is based on the genetic concept of induced essentiality, where functional interactions that occur in response to oncogene addiction create a dependency on another gene.

To identify genetic dependencies in *NRAS*-mutant melanoma, whole-genome CRISPR/Cas9 dropout screens were conducted in a collection of NZM melanoma cell lines that were established from NZ melanoma patients and maintained at physiological oxygen conditions (5% O_2). We have stably transduced 6 *NRAS*-mutant and 7 *NRAS*-wild-type NZM cell lines with a Cas9 nuclease and the full-genome Brunello lentiviral sgRNA library, and the knockout libraries have been screened for up to 35 days. Changes in the abundances of PCR-amplified sgRNA sequences have been quantitated with next-generation sequencing. This was combined with the CRISPR-Cas9 screening data using the Avana library from an additional 28 melanoma cell lines, available on the Cancer Cell Line Encyclopaedia (CCLE) database and, analysed using BAGEL bioinformatics software packages. Candidate genes that are deleterious to the fitness of each *NRAS*-mutant cell line when knocked out, will be further validated as essential genes for *NRAS*-mutant melanoma cells through *in vitro* and *in vivo* individual gene knockout studies. The identification of genetic dependencies alongside *NRAS* mutations may provide potential new drug targets for the development of therapeutic strategies for the treatment of *NRAS*-mutant melanoma.

CT25: *Ex vivo* gene therapy for epidermolysis bullosa; severe skin fragility disorders

Hunt, J.[1], du Rand, A.[1], Samson, C.[1], Meidinger, S.[1], Lehnert, K.[1], Dunbar, R.[1], Knapp, D.[2], Mahon, C.[4], Purvis, D.[3], Feisst, V. [1], Sheppard, H.[1]

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The development of specific, efficient, and versatile genome editing tools such as CRISPR/Cas9 has accelerated the field of genome editing. However, the progression of these technologies into personalised therapeutic applications are hindered by the low efficacy of correction and safety concerns surrounding 'off-target' genotoxicity. This research aims to correct patient-specific mutations in human skin cells and develop a low-risk, proof-of-principle genome editing application. Epidermolysis bullosa (EB) encompasses a group of monogenic skin disorders characterised by severe blistering in response to minor friction. *Ex vivo* gene therapy using gene-engineered skin may significantly decrease disease burden and provide a novel cure for people with EB.

We hypothesise that *ex vivo* gene therapies for recessive dystrophic EB will be most effective when delivered as bilayered skin equivalents, so will require the genetic correction of patient epidermal keratinocytes and dermal fibroblasts. Using CRISPR/Cas9 delivered as ribonucleoproteins and short ssDNA repair templates, we achieve up to 50% allele-specific homology-directed repair (HDR) in epidermal keratinocytes. Preliminary data indicates similar rates of HDR in dermal fibroblasts. To ensure the safety of CRISPR/Cas9 editing we intend to analyse editing outcomes, including structural variants, in pooled heterogeneous DNA using Oxford Nanopore Technology sequencing. We will assess the functional consequences of gene correction by digital-droplet PCR (mRNA) and immunocytochemistry (protein) assays. Finally, we have developed novel methods to generate bilayered skin equivalents to help facilitate their transition into the clinic. We utilise both commercially available skin substitutes and a novel synthetic mesh designed for burns patients to generate robust bilayered skin sheets. These methods replicate the EB blistering phenotype so may act as a functional model of gene correction. Overall, this research aims to pave the way for clinical-grade therapeutic genome editing in New Zealand.

CT26: Disrupting the oligomeric structure of peroxiredoxins in cancer cells using CRISPR

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Peroxiredoxins are a family of proteins that protect cells from oxidative stress by consuming hydroperoxides[1,2]. Peroxiredoxins have complex oligomeric structures that are altered by oxidation, and they appear to regulate signalling pathways through specific protein-protein interactions[2-6]. Using CRISPR technology we have created knockouts of two cytoplasmic members of the peroxiredoxin family[4], and also created a knock-in mutation that disrupts the oligomeric structure of peroxiredoxin 2, while retaining its ability to consume hydrogen peroxide, albeit more slowly. Intriguingly, the structural mutant was more damaging when expressed in Jurkat T-lymphoma cells than complete removal of the protein, suggesting a gain-of-function. We are currently undertaking a complete characterisation of cells expressing the mutant protein in order to understand its mechanism of action.

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CT27: Exploring Gene Editing of Aryl Hydrocarbon Receptor Gene in Primary Wild Type and Leukaemia Bone Marrow Cells Using HDR Pathway of CRISPR-Cas System

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Acute leukaemia is a devastating disease with poor prognosis. It is initiated by leukaemia stem cells (LSCs), which are able to survive treatment and often drive relapse. Targeting a signalling pathway that is common to most LSCs could be a novel treatment strategy. The aryl hydrocarbon receptor (AHR) pathway was recently shown to be critical for the self-renewal properties of normal haematopoietic stem cells (HSCs). Our aim is to study the effects of genetic manipulation of the AHR pathway *in vitro* and *in vivo* in a leukaemia model. Previously, first syngeneic leukaemia models in immunocompetent mice in New Zealand were established in our group. Now, one of them, CA-MF, we use for genetic manipulation of the *Ahr* using homology-directed DNA repair (HDR) pathway of the CRISPR gene editing technology. In order to knock out *Ahr* gene we designed several guide RNAs (# 1 -3) that target exon 2 of the *Ahr* and two HDR templates to be knocked-in (KI) in that region. We aimed to target both alleles with two distinct templates: bearing mCherry or blue fluorescent protein (BFP). We generated these using the Guide-it long ssDNA production system and delivered them to primary bone marrow (BM) cells along with Cas9 protein and guide RNAs as a ribonucleoprotein complex (RNP) using electroporation. RNP delivery to the primary bone marrow cells, either leukaemia or healthy wild type, via electroporation was highly challenging (high death rates, >95% following electroporation). Cas9 *in vitro* assay confirmed efficiency of DNA editing. We optimised electroporation (DN-100 and CM-137, with the latter program producing the best cell viability). In BM cells two combinations of the guides (#1+3 and #2+3) were found to be efficient at DNA cleavage, however, no KI efficiency was detected. Overall, we achieved cleavage of DNA with our system and further optimisation is required.

CT28: Towards the development of *ex vivo* gene therapy for the severe group of fragile skin disorders epidermolysis bullosa

du Rand, A.[1], Hunt, J.[1], Samson, C.[1], Meidinger, S.[1], Mahon, C.[4], Dunbar, R.[1], Purvis, D.[2], Feisst, V.[1], Sheppard, H.[1]

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Genome editing tools such as CRISPR/Cas9 hold tremendous promise as curative therapies for a range of human diseases. For epidermolysis bullosa (EB), a devastating group of monogenic skin blistering disorders, these technologies have already demonstrated encouraging therapeutic potential. However, progression into the clinic has been hampered by low rates of gene correction obtained via homology-directed repair (HDR) and safety issues relating to off-target mutagenesis. In our research, we aim to address these concerns by establishing low risk, highly efficient *ex vivo* genome editing protocols to correct causative EB mutations in the two major human skin cell types: dermal fibroblasts and epidermal keratinocytes. Following CRISPR/Cas9 editing, we intend to use these cells to engineer gene corrected skin substitutes that may substantially decrease disease severity and provide lifesaving treatments for people with EB. We first aim to investigate an exon skipping approach to remove in-frame, mutation harbouring exons. This strategy bypasses the requirement for low efficiency HDR-mediated repair, and instead, relies on the highly efficient non-homologous end joining (NHEJ) repair pathway. Employing this strategy, we have achieved exon deletion rates of >95% in dermal fibroblasts and epidermal keratinocytes. Additionally, we intend to trial Cas9 nickase, a modified CRISPR/Cas9 system which carries a lower risk of off-target toxicity. To determine the functional consequences of CRISPR/Cas9 editing on mRNA and protein, we will use a range of techniques including droplet digital PCR (ddPCR), flow cytometry, immunocytochemistry and western blot. We plan to use Oxford nanopore sequencing to verify the safety of our editing strategies. Lastly, using novel methods developed inhouse, we will grow gene-corrected, bilayered skin substitutes to facilitate the translation of these therapies into the clinic. Overall, the goal of our research is to establish clinical-grade therapeutic genome editing in New Zealand.

CT29: Identification of CRISPR-Cas and novel phage defense systems to expand our molecular toolkit

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CRISPR-Cas systems are one of many types of antiviral defence systems that have evolved in bacteria to protect against invasion by viruses and mobile genetic elements. The discovery and characterisation of antiviral defence systems has led to the development of several ground-breaking molecular tools, such as Cas9 for genome editing. The ongoing discovery and characterisation of new CRISPR-Cas systems has further expanded the biotechnological use-cases for Cas proteins, including for CRISPR-based diagnostics (e.g. Cas12 and Cas13). It is likely that the discovery and characterisation of other types of defence systems will reveal additional novel enzymes with biotechnological applications. In the past four years, the number of known types of defence systems has increased more than 5–fold, and the discovery of new defence systems is quickly outpacing the development of tools that make use of these insights. To address this, we developed the Prokaryotic Antiviral Defence LOCator (PADLOC), a bioinformatics tool and webserver (padloc.otago.ac.nz) that enables the detection of >160 types of diverse defence systems in microbial genomes, including 27 subtypes of CRISPR-Cas systems. PADLOC provides a comprehensive view of the defence arsenal of any microbe and can be used to identify candidate systems to study as new molecular tools. Here, we describe our use of PADLOC to perform large-scale defence system identification in more than 200,000 archaeal and bacterial genomes and probing the results to discover multiple new types of defence systems. We experimentally demonstrate the activity of novel defence systems encoding diverse Helicases, Methylases, DNases, RNases, ATPases, toxins, and other proteins with unique domains of unknown functions. The data presented here expands on the ever-growing spectrum of microbial defence systems against viruses, and uncovers new systems with potential as novel biotechnological agents.