## Genetic Modification of Haematopoietic Stem Cells. Methods and Protocols

*C. Baum ed. Heidelberg: Springer, 2009.*
*ISBN 978-1-58829-980-2. 490 pp. £67.99.*

This comprehensive book presents detailed protocols for genetic modification of haematopoietic stem cells (HSCs) and is written by the pre-eminent researchers in the field. *Ex vivo* gene therapy is a fast-emerging area with clinical trials already underway for some diseases. This book fills an important gap both for the basic and the clinical scientist, as well as providing guidance on the regulatory hurdles likely to be encountered in this highly specialised area.

The first few chapters deal with the isolation and enrichment of HSCs from various sources, followed by viral vector design and production and issues arising from transferring from small- to large-scale vector production. There are also protocols covering the transduction of human and mouse HSCs, T lymphocytes, natural killer and dendritic cells with viral (lentiviral and retroviral) and non-viral (DNA-based transposons) vectors and the *in vivo* and *ex vivo* application of gene transfer into HSCs.

The book includes numerous protocols for biosafety testing of cells and vectors prior to clinical application and focuses on the importance of rigorous quality control, good manufacturing practice and the risks associated with potential stem cell gene therapies. Many of these protocols provide the basis for the biosafety testing currently required by the regulators. Therefore, protocols for the detection of replication-competent virus, insertional mutagenesis, leukaemias, clonal tracking, insertion site analysis and copy number are included. This book is also a good source of information on US and EU regulations on the clinical use of HSCs that have been genetically modified, although, due to the constant flux of regulatory body requirements, this section will rapidly become dated.

Our only criticism would be with the organisation of the chapters, as these are not grouped very systematically. Design and production of vectors comes after transduction of cells, for example. The organisational chart presented in the preface has a much clearer and more systematic order of presentation than the actual chapter order and we suggest the reader follows the chart in the preface.

Nevertheless, this excellent book is a vital resource for anyone involved in developing a stem cell gene therapy approach for curing genetic disease, and provides an essential technical manual for anyone wishing to genetically modify haematopoietic stem cells.

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## Haematology Morphology Training CD-ROM

*A. Blann, R. Clarke, D. Gurney, A. Henley, L. Hill, P. Holtom, N. Humble, I. Jennings, S. Marwah, G. Moore, A. Roderick on behalf of the Institute of Biomedical Science. London: IBMS, 2009.*

Changes in working practice and the use of increasingly sophisticated haematology analysers have led to a reduction in the number of peripheral blood films needing microscopic review, with a loss of opportunity for biomedical scientists to gain skills in blood cell morphology. Reduced staffing and shift work have had a significant impact on training initiatives within the modern laboratory. Advances in media technology mean that a training CD of this type will be most welcome. A well thought out digital morphology CD might be superior to the traditional printed blood cell atlas as a teaching aid, allowing access to a larger range of images and interactive features.

The CD is presented as a photo-gallery with associated text. The overall structure is logical, divided into five sections covering the main areas of concentration when examining a blood film. It includes the important topics of how to view a blood film and examples of interfering artefacts. However, there are a number of criticisms that will limit the application of this publication.

While it is noted that staining quality and methods differ between laboratories and the viewing quality of the CD will be affected by the set up of the local PC monitor, the quality of the imaging presented is disappointing. The section on 'artefacts and rarities' hardly seems necessary when many slides in earlier sections have already demonstrated features such as fixation artefacts. The poor imaging and inconsistent staining was unexpected, given that the CD is supported by a commercial company that markets an automated slide imaging system capable of producing images of excellent quality.

The content of some sections is incomplete. For example, the section on how to view a slide would benefit from instruction on how to make a blood film and a summary of the main stains used in haematology. The decision as to what images to include in a photo-gallery is difficult but a guide such as this should contain sound images of normal and commonly encountered abnormal cells. A section (just one or two slides) on cell lineage maturation at the beginning might have helped the logical flow, especially as there are only a few immature cells on the various images of abnormal conditions. The platelet section should include additional slides showing thrombocytopenia and giant platelets, as well as some simple platelet abnormalities. It is good that the CD advises at the outset that it is viewed alongside an experienced morphologist as some images are highly atypical (e.g., image 3 in the myeloma section), and will need a more experienced eye to understand features that are not explained in the text. It is also a shame that the authors did not incorporate more annotations on the images (e.g., measurement of cell size when discussing red cell size), especially as different magnifications have been used to obtain the images, or pointing out the keratocyte in the image of normal red cells. Annotations would also have been useful for the macrocytic and microcytic red cell images and to highlight the abnormal red cells in the image of the normal basophil.

The accompanying text may be misleading in some instances. An image of acute promyelocytic leukaemia, with no blast cells present, is accompanied by text that contains the advice that "with experience comes the ability to

recognise different types of blasts". Nowhere in the text, however, is there mention of the most basic features of different types of blast, nor the necessity for further testing (e.g., immunophenotyping or cytochemistry) for full identification. A slide of chronic myeloid leukaemia (CML) is accompanied by a description including the term 'many primitive cells' – CML is known for many immature but developing cells, predominantly myelocytes and neutrophils. 'Primitive' may imply blast cells, which are a minority in chronic-phase CML. This presumably is where the experienced trainer is expected to fill in the gaps. In another instance an image of a polychromatic cell is identified as a reticulocyte and is presented with a metamyelocyte, as if the two are necessarily found together; this would have been better illustrated with an image of a supravitally stained reticulocyte accompanied by a good image of polychromasia. There is a depiction of macrocytes in liver disease but with no mention of target cells, stomatocytes or thrombocytopenia usually associated with that condition. The occasional mistake in the spelling of scientific terms (e.g., 'reticulocute' instead of reticulocyte) gives an impression of a lack of care taken over the preparation of the text; the use of hyper- and hypochromasia, rather than the more usual hyper- and hypochromia, should also be reviewed.

The final section of 'artefacts and rarities' seems bizarre as these two have nothing in common. The inclusion of malarial parasites in this section was a surprise as malaria is neither an artefact nor a rarity in much of the world. Again, there are some errors in the text: the caption with slide 44 states that the "small dots are schizonts", when in fact they are James or Schuffners dots. The lack of a separate section for malaria and other blood parasites is a lost opportunity. Blood parasites always fascinate those in training, and a teaching aid of this nature could provide an excellent reference tool in this area, with potential for use in the developing world.

Overall, this CD represents an excellent concept and could provide an invaluable teaching aid for biomedical scientists. In the current economic climate, this is a cost-effective way of providing training information both for trainer and trainee in the haematology laboratory. Our profession is crying out for this sort of aid and the team should be congratulated; however, this is a work in progress and a second edition with improved images would be welcomed.

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**CPD**

# Oxidative and nitrosative stress in β-cell apoptosis: their contribution to β-cell loss in type 1 diabetes mellitus

Watson D, Loweth AC. *Br J Biomed Sci* 2009; **66**: 208–15.
Assessment No: **129909** (please enter on JBL card or on website).

| | | | |
|---|---|---|---|
| A | Type 1 diabetes mellitus (DM) is classified as an autoimmune disease. | TRUE | FALSE |
| B | The Stockholm model has been used to describe the processes involved in the coordination of immune cells in type 1 DM. | TRUE | FALSE |
| C | Production of α-cell-targeted autoantibodies correlates with deteriorating glycaemic control. | TRUE | FALSE |
| D | In gene signalling networks stimulated by pro-inflammatory cytokines, around 50 genes are dependent on NF-κB activation. | TRUE | FALSE |
| E | In RIN-r β-cells, cytokine-derived nitric oxide (NO) is a primary contributor to cell loss. | TRUE | FALSE |
| F | Exposure of rat islets to interleukin (IL)-1β inhibits insulin secretion. | TRUE | FALSE |
| G | Cytokine-derived NO may sensitise human islets to T cells via the up-regulation of Fas. | TRUE | FALSE |
| H | Islet transplantation represents a promising therapy for the treatment of type 1 DM. | TRUE | FALSE |
| I | Micro-encapsulation has failed to protect transplanted islets from immune attack. | TRUE | FALSE |
| J | Following cytokine treatment, both iNOS and MnSOD are down-regulated. | TRUE | FALSE |
| K | Over-expression of Bcl-2 can inhibit cytokine-induced mitochondrial dysfunction and apoptosis. | TRUE | FALSE |
| L | Islet β-cells are one of the most susceptible cell types to ER stress. | TRUE | FALSE |
| M | The small molecule inhibitor salubrinal is well tolerated by β-cells. | TRUE | FALSE |
| N | Manganese superoxide dismutase is only a minor antioxidant enzyme. | TRUE | FALSE |
| O | Human islet clusters can comprise up to 5000 cells. | TRUE | FALSE |
| P | Human islets are highly sensitive to peroxynitrite toxicity. | TRUE | FALSE |
| Q | Activation of RyR may contribute to glucose-stimulated insulin secretion. | TRUE | FALSE |
| R | Expression of CHOP decreases after activation of an ER stress response. | TRUE | FALSE |
| S | Cytokine combinations can induce accumulation of toxic intracellular ROS and RNS. | TRUE | FALSE |
| T | Bax protein is a member of the Bcl-2 family. | TRUE | FALSE |

Deadline for submission: Thursday 1 April 2010