Opt!STEM
Optimization of Stem cell Therapy for degenerative Epithelial and Muscle diseases

2007 - 2013

EndoStem

News

www.optistem.org www.endostem.eu

April 2010 – Contents

1. YOUNG SCIENTIST NETWORK NEWS (VIRTUAL AND PHYSICAL MEETINGS):

ONLINE MINI CONGRESS SERIES KICKS OFF: INNOVATIVE IMAGING TECHNIQUES

20TH MAY FROM 9.30 TO 12.00

You will have a comprehensive introduction to several imaging techniques. The confirmed speakers and talks are the following:

9.30 -10.00: **Gisela Kuhn**, ETH Zurich. „Technique and applications of micro-computed tomography “

10.00 – 10.30: **David Margolis**, University Zurich. “In vivo two-photon imaging of neuronal activity: from networks to behavior"

10.30 – 10.45: Break

10.45 – 11.15: **Marie-Hélène Boudrias**, University College London. “Basis of the BOLD signal in fMRI and application”

11.15 - 11.45: **Jeronimo Blanco**, CSIC Barcelona. “Non-invasive bioluminescence imaging for cell therapy”

11.45 – 12.00: Discussion and feedbacks on this first online event

To make sure there are not too many technical issues, we have to restrict the attendance to this first tutorial to a maximum of 25 computers. But there could be more than one person in front of a computer in your lab, thus allowing the number of participants to be above 25.

To register, please enter your name(s) in the following Doodle session, first come, first served!

http://dandoandcolucci.doodle.com/m7pckckrfzbzk8au

JUNE the week of the 14th to the 18th– precise date to be confirmed

Karen English from Kathryn Wood's lab will talk about Mesenchymal stem cell modulation of Natural killer T cells in vivo?

2nd speaker to be confirmed

June 30th to July 2nd - TOPEA YOUNG SCIENTISTS CONGRESS – BARCELONA

Middle of July – Optistem and Endostem: Project team web meetings

SEPTEMBER – DATES AND TOPICS TO BE CONFIRMED:

OptiStem & EndoStem young scientist seminar (2 x 20' speakers) : these are web seminars as you know them, run jointly with EndoStem partners

2. TOPEA YOUNG SCIENTIST MEETING

Young scientists meeting in July 2010 in Barcelona, for and by young scientists

MEETING PROGRAMME CAN BE FOUND ON PAGE 4

3. STEMDIRECT - A SMART CONTACT DATABASE FOR STEM CELL SCIENTISTS IN EUROPE

StemDirect is:

- Free to use – no registration required to view or search
- Monitored and maintained – only relevant profiles are published, and details are kept up-to-date
- Useful for researchers, communicators and project administrators/managers for:
    - Finding collaborators
    - Raising your own profile
    - Identifying colleagues for exchange of ideas
- Includes profiles of the following: Group Leaders, Lab Managers, Post-Docs, Research Assistants, Scientific Officers, Science Communicators, Network Managers/Administrators, Research Project Manager/Administrator - new contacts can submit their details for inclusion in the database by completing the registration from online.

4. EUROSYSTEM OPEN CALL FOR MEMBERS TO JOIN ITS STEM CELL GROUP

The FP7 consortium EuroSyStem is running a call for members to join its European Stem Cell Group. As you may remember, the group was founded by EuroSyStem in 2009 and we ran a similar call for members then. The aim is to bring together fundamental stem cell scientists from across Europe, to encourage scientific exchange and collaboration. Members will have access to specialist data management tools, training and meetings.

MEMBERSHIP BENEFITS
Members of the European Stem Cell Group will become Associate PIs of EuroSyStem and will have access to: . Annual closed meeting . Exchange of ideas with other experts . Work groups for pre-publication discussion . Bursary scheme for exchanges visits . Specialist data management and analysis tools . Training courses

WHO CAN APPLY
Independent group leaders working in an EU/EEA country, with an active research programme focused on stem cell biology (any organism) may apply to join. Applications from emerging investigators (with fewer thanmfive years' experience as an independent group leader) and researchers in new member states are particularly welcome. See attached document for full criteria.

APPLICATION

Deadline: 9th July 2010. Further information on membership criteria and application form: http://www.eurosystemproject.eu/news

AVANCES

Opt!STEM
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REPORTING:

The periodic report (activity and financial) has now been approved – the EC will soon be making the next payment, so funds should be with you soon.

EndoStem

ADMIN ISSUES

The grant agreement has been signed and countersigned. The A forms have been received in Inserm and the funds have arrived with them. As soon as the A forms are counter signed the funds will be transferred

Please do not forget the need for **timesheets**, they are annoying but they are needed.

CONTACT DETAILS

Please make sure that Revital Rattenbach (RTD coordinator) receives the contact details for all personnel linked to the Endostem project, specially the young scientists.

PUBLICATIONS AND ACCEPTED MANUSCRIPTS:

Please make sure you now acknowledge the support of the European Commission and Endostem in all publications related to the work. Standard text to use is:

This work has benefited from research funding from the European Community's Seventh Framework Programme in the project FP7-Health – 2009 ENDOSTEM 241440 (*Activation of vasculature associated stem cells and muscle stem cells for the repair and maintenance of muscle tissue*)

TOPEA DEGENERATIVE DISEASE/REGENERATIVE MEDICINE MEETING – AGENDA (SO FAR – WE ARE ALSO INTEGRATING IN OUTREACH TRAINING):

TOPEA MEETING – Barcelona Biomedical Research Park
June 30th to July 2nd 2010

UPDATED AGENDA

29th June 2010

Afternoon	You should plan to arrive sometime in the afternoon

30th June 2010

9.30 – 10.00	**Registration and coffee**
10.00 – 12.30	**Muscle regeneration** **Antonella Biondo** (Giuilo Cossu) *Engineered skeletal muscle tissue for in vitro reconstitution and in vivo transplantation* **Ombretta Guardiola** (Gabriella Minchiotti) *A novel role for Cripto in post-natal skeletal muscle regeneration through modulation of TGFb family signaling pathways* **Joanne Tonkin** (Nadia Rosenthal) *IGF-1 Modulation of Macrophage Activity in Skeletal Muscle regeneration* **Arianna Dellavalle** (Giuilo Cossu) *Vessels associated cells fuse with developing skeletal muscle fibers and enter the satellite cell compartment during mouse post-natal life* **Emanuele Azzoni** (Silvia Brunelli) *Embryonic endothelial derived progenitors contribute to skeletal and smooth muscle development and participate to muscle regeneration*
12.30 – 14.00	*Light lunch*

13.30 – 15.00 **Bone regeneration**

Arlyng Gyveth, González Vázquez (Josep Planell)
Extracellular Calcium's Role on the Progenitor Bone Marrow Cells Behavior. Relation in Bone Tissue Engineering

Georg Alexander FEICHTINGER (Heinz Redl)
Inducible BMP2/BMP7 Co-Expression System for Osteoinduction

Edgar B. Montufar (Maria-Pau Ginebra)
Injectable Hydroxyapatite Foams for Bone Regeneration

15.00 – 15.30 *Coffee break*

15.30 – 18.00 **Endothelial regeneration**

Kathrin Pütsch (Miltenyi Biotech)
Enrichment and Enumeration of circulating endothelial Cells

Veronica Sacchi (Andrea Banfi)
Dose- and time-dependent angiogenesis by controlled delivery of matrix-bound VEGF

Silvia Reginato (Andrea Banfi)
PDGF-BB regulates the switch between normal and aberrant angiogenesis and accelerates vessel stabilization

Fabrizio Orsenigo (Elizabetta Dejana)
Zebrafish as a model system to test biomaterials

Esra Guc (Melody Swartz)
Fundamental Characterization of Lymphangiogenesis and Angiogenesis

18.00 – 21.00 *Free time*

21.00 *Dinner*

1st July 2010

10.00 – 11.00 — **Epithelial regeneration**

Melissa Maggioni (Yann Barrandon)
In vitro *characterization of clonogenic human thymic epithelial cells*

Olaf Hardt (Miltenyi Biotech)
Isolation of (stem) cell populations from heart, liver, and skin tissue using semi-automated dissociation and magnetic cell separation

11.00 – 12.00 — **Imaging in tissue regeneration**

Susanne Wolbank (Heinz Riedl)
Non-invasive in vivo tracking of fibrin degradation by fluorescence imaging

Laura Nebuloni (Ralph Muller)
In vivo micro-computed tomography of the vascular network for quantification of angiogenesis in tissue regeneration

12.00 – 16.00 — *Lunch and free time*

16.00 – 18.30 — **Biomaterials development**

Kandice Levental (Carsten Werner)
Optimization of starPEG-heparin hydrogels for inducing angiogenesis

Oommen P. Varghese (Jons Hilborn)
Hyaluronic acid hydrogels for tissue engineering and gene delivery

Steffen Cosson (Matthias Lütolf)
Biomolecular gradients arrays on biomimetic hydrogels for cell-based assays

Jennifer Patterson (Jeff Hubbell)
Enhancing the Proteolytic Degradation of Molecularly Engineered PEG Hydrogels to Improve Angiogenesis

Adrian Ranga (Matthias Lütolf)
Modulating the PEG hydrogel microenvironment via novel enzyme activity-based mechanisms

18.30 – 20.00 — **POSTER SESSION** *with Drinks & Snacks*

21.00 — *Dinner*

2nd July 2010

10.00 – 11.30 Neurological regeneration

Jessica Kwok (James Fawcett)
Extracellular matrix molecules in controlling CNS plasticity

Linard Filli (Martin Schwab)
Neuronal regeneration and plasticity after spinal cord injury

Michèle Hubli (Volker Dietz)
Spinal reflexes: a possible marker for walking ability after spinal cord injury

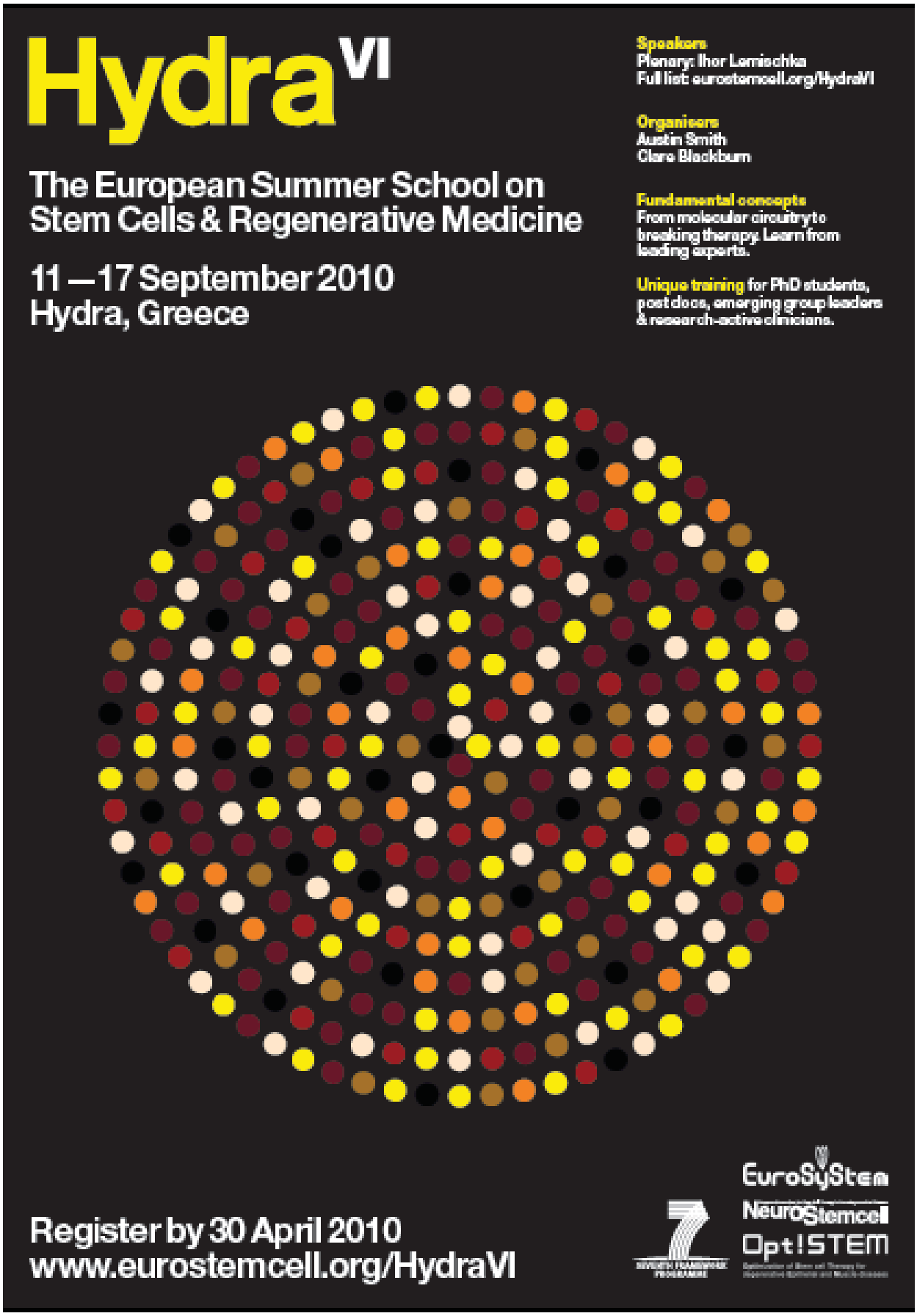
11.30 – 12.30 Clinical translation

Marie-Hélène Boudrias (Nick Ward)
Age related changes in motor system connectivity assessed with Dynamic Causal Modelling

Christine Kubiak (ECRIN)
title to be confirmed

13.00 End of meeting

HYDRA MEETING



FRIC: OPEN FUNDING CALLS

To access the **86** pages of open calls that are identified in the new monthly publication

'Money'

Click on the following hyperlink

OPEN FUNDING CALLS

and click on the 04 2010 edition

or post the link below into your web browser

http://pro.en.mayetic.com/QuickPlace/dandoandcolucci/Main.nsf/h_AD3E5D918B25566DC12576BD0037D1C2/A6E375DA2F6AE21BC12577180035A062/?OpenDocument

SAPIENZA: LITERATURE UPDATES FROM PUBMED

Key words used:
'stem cells and muscle' 'stem cells and epithelia' 'muscle cell biology' 'epithelial cell biology' 'stem cell clinical trials' 'stem cell transplantation' 'nitric oxide and muscle' 'HMGB' 'HDAC' 'Cripto' 'myostatin inhibitors' 'biomaterial drug delivery'

STEM CELLS AND MUSCLE

1. Severe X-linked mitochondrial encephalomyopathy associated with a mutation in apoptosis-inducing factor.

Am J Hum Genet. 2010 Apr 9;86(4):639-49. Epub 2010 Apr 1.

Ghezzi D, Sevrioukova I, Invernizzi F, Lamperti C, Mora M, D'Adamo P, Novara F, Zuffardi O, Uziel G, Zeviani M.

We investigated two male infant patients who were given a diagnosis of progressive mitochondrial encephalomyopathy on the basis of clinical, biochemical, and morphological features. These patients were born from monozygotic twin sisters and unrelated fathers, suggesting an X-linked trait. Fibroblasts from both showed reduction of respiratory chain (RC) cIII and cIV, but not of cI activities. We found a disease-segregating mutation in the X-linked AIFM1 gene, encoding the Apoptosis-Inducing Factor (AIF) mitochondrion-associated 1 precursor that deletes arginine 201 (R201 del). Under normal conditions, mature AIF is a FAD-dependent NADH oxidase of unknown function and is targeted to the mitochondrial intermembrane space (this form is called AIF(mit)). Upon apoptogenic stimuli, a soluble form (AIF(sol)) is released by proteolytic cleavage and migrates to the nucleus, where it induces "parthanatos," i.e., caspase-independent fragmentation of chromosomal DNA. In vitro, the AIF(R201 del) mutation decreases stability of both AIF(mit) and AIF(sol) and increases the AIF(sol) DNA binding affinity, a prerequisite for nuclear apoptosis. In AIF(R201 del) fibroblasts, staurosporine-induced parthanatos was markedly increased, whereas re-expression of AIF(wt) induced recovery of RC activities. Numerous TUNEL-positive, caspase 3-negative nuclei were visualized in patient #1's muscle, again indicating markedly increased parthanatos in the AIF(R201 del) critical tissues. We conclude that AIF(R201 del) is an unstable mutant variant associated with increased parthanatos-linked cell death. Our data suggest a role for AIF in RC integrity and mtDNA maintenance, at least in some tissues. Interestingly, riboflavin supplementation was associated with prolonged improvement of patient #1's neurological conditions, as well as correction of RC defects in mutant fibroblasts, suggesting that stabilization of the FAD binding in AIF(mit) is beneficial.

2. Nerve growth factor promotes cardiac repair following myocardial infarction.

Circ Res. 2010 Apr 16;106(7):1275-84. Epub 2010 Apr 1.

Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, Spillmann F, Campesi I, Madeddu P, Quaini F, Emanueli C.

RATIONALE: Nerve growth factor (NGF) promotes angiogenesis and cardiomyocyte survival, which are both desirable for postinfarction myocardial healing. Nonetheless, the NGF potential for cardiac repair has never been investigated.

OBJECTIVE: To define expression and localization of NGF and its high-affinity receptor TrkA (tropomyosin-related receptor A) in the human infarcted heart and to investigate the cardiac roles of both endogenous and engineered NGF using a mouse model of myocardial infarction (MI).

METHODS AND RESULTS: Immunostaining for NGF and TrkA was performed on heart samples from humans deceased of MI or unrelated pathologies. To study the post-MI functions of endogenous NGF, a NGF-neutralizing antibody (Ab-NGF) or nonimmune IgG (control) was given to MI mice. To investigate the NGF therapeutic potential, human NGF gene or control (empty vector) was delivered to the murine periinfarct myocardium. Results indicate that NGF is present in the infarcted human heart. Both cardiomyocytes and endothelial cells (ECs) possess TrkA, which suggests NGF cardiovascular actions in humans. In MI mice, Ab-

NGF abrogated native reparative angiogenesis, increased EC and cardiomyocyte apoptosis and worsened cardiac function. Conversely, NGF gene transfer ameliorated EC and cardiomyocyte survival, promoted neovascularization and improved myocardial blood flow and cardiac function. The prosurvival/proangiogenic Akt/Foxo pathway mediated the therapeutic benefits of NGF transfer. Moreover, NGF overexpression increased stem cell factor (the c-kit receptor ligand) expression, which translated in higher myocardial abundance of c-kit(pos) progenitor cells in NGF-engineered hearts.

CONCLUSIONS: NGF elicits pleiotropic beneficial actions in the post-MI heart. NGF should be considered as a candidate for therapeutic cardiac regeneration.

3. Development of a rapid culture method to induce adipocyte differentiation of human bone marrow-derived mesenchymal stem cells.

Biochem Biophys Res Commun. 2010 Apr 2;394(2):303-8. Epub 2010 Mar 3.

Ninomiya Y, Sugahara-Yamashita Y, Nakachi Y, Tokuzawa Y, Okazaki Y, Nishiyama M.

Human mesenchymal stem cells (hMSCs) derived from bone marrow are multipotent stem cells that can regenerate mesenchymal tissues such as adipose, bone or muscle. It is thought that hMSCs can be utilized as a cell resource for tissue engineering and as human models to study cell differentiation mechanisms, such as adipogenesis, osteoblastogenesis and so on. Since it takes 2-3weeks for hMSCs to differentiate into adipocytes using conventional culture methods, the development of methods to induce faster differentiation into adipocytes is required. In this study we optimized the culture conditions for adipocyte induction to achieve a shorter cultivation time for the induction of adipocyte differentiation in bone marrow-derived hMSCs. Briefly, we used a cocktail of dexamethasone, insulin, methylisobutylxanthine (DIM) plus a peroxisome proliferator-activated receptor gamma agonist, rosiglitazone (DIMRo) as a new adipogenic differentiation medium. We successfully shortened the period of cultivation to 7-8days from 2-3weeks. We also found that rosiglitazone alone was unable to induce adipocyte differentiation from hMSCs in vitro. However, rosiglitazone appears to enhance hMSC adipogenesis in the presence of other hormones and/or compounds, such as DIM. Furthermore, the inhibitory activity of TGF-beta1 on adipogenesis could be investigated using DIMRo-treated hMSCs. We conclude that our rapid new culture method is very useful in measuring the effect of molecules that affect adipogenesis in hMSCs.

4. Sox2 transduction enhances cardiovascular repair capacity of blood-derived mesoangioblasts.

Circ Res. 2010 Apr 16;106(7):1290-302. Epub 2010 Feb 25.

Koyanagi M, Iwasaki M, Rupp S, Tedesco FS, Yoon CH, Boeckel JN, Trauth J, Schütz C, Ohtani K, Goetz R, Iekushi K, Bushoven P, Momma S, Mummery C, Passier R, Henschler R, Akintuerk H, Schranz D, Urbich C, Galvez BG, Cossu G, Zeiher AM, Dimmeler S.

RATIONALE: Complementation of pluripotency genes may improve adult stem cell functions.

OBJECTIVES: Here we show that clonally expandable, telomerase expressing progenitor cells can be isolated from peripheral blood of children. The surface marker profile of the clonally expanded cells is distinct from hematopoietic or mesenchymal stromal cells, and resembles that of embryonic multipotent mesoangioblasts. Cell numbers and proliferative capacity correlated with donor age. Isolated circulating mesoangioblasts (cMABs) express the pluripotency markers Klf4, c-Myc, as well as low levels of Oct3/4, but lack Sox2. Therefore, we tested whether overexpression of Sox2 enhances pluripotency and facilitates differentiation of cMABs in cardiovascular lineages.

METHODS AND RESULTS: Lentiviral transduction of Sox2 (Sox-MABs) enhanced the capacity of cMABs to differentiate into endothelial cells and cardiomyocytes in vitro. Furthermore, the number of smooth muscle actin positive cells was higher in Sox-MABs. In addition, pluripotency of Sox-MABs was shown by demonstrating the generation of endodermal and ectodermal progenies. To test whether Sox-MABs may exhibit improved therapeutic potential, we injected Sox-MABs into nude mice after acute myocardial infarction. Four weeks after cell therapy with Sox-MABs, cardiac function was significantly improved compared to mice treated with control cMABs. Furthermore, cell therapy with Sox-MABs resulted in increased number of differentiated cardiomyocytes,

endothelial cells, and smooth muscle cells in vivo.

CONCLUSIONS: The complementation of Sox2 in Oct3/4-, Klf4-, and c-Myc-expressing cMABs enhanced the differentiation into all 3 cardiovascular lineages and improved the functional recovery after acute myocardial infarction.

5. Activation of adventitial fibroblasts in the early stage of the aortic transplant vasculopathy in rat.

Transplantation. 2010 Apr 27;89(8):945-53.

Ji J, Xu F, Li L, Chen R, Wang J, Hu WC.

BACKGROUND: Transplant vasculopathy (TV) is the most significant obstacle to long-term success of organ transplantation. Increasing attention has been paid to the role of adventitia in vascular diseases. We evaluated the role of adventitial fibroblasts in the development of TV.

METHODS: Thoracic aortas from Sprague-Dawley (SD) rats transplanted into the abdominal aortas of Wistar rats worked as allografts, and isografts (SD to SD) were control. Grafts were removed on days 3, 7, and 14 for histologic, morphometric, and immunohistochemical detection of vimentin, alpha-smooth muscle actin, Ki-67, CD3, transforming growth factor-beta1 (TGF-beta1), monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-7 (MMP-7), and quantitative real-time reverse transcriptase polymerase chain reaction for TGF-beta1, MCP-1, MMP-7, tumor necrosis factor-alpha, and interleukin-1beta.

RESULTS: In the allografts, neointima thickness and neointima/media thickness ratios were slightly increased at 7 days and significantly increased at 14 days. Immunostaining of vimentin and alpha-smooth muscle actin showed adventitial fibroblasts activation and differentiation into myofibroblasts. Ki-67-positive nuclei were observed in the adventitia 3 days after allografting and subsequently in the neointima. No more than 4% CD3-positive cells were found in adventitia in all the groups. Compared with isografts, TGF-beta1, MMP-7, and MCP-1 were expressed in the adventitia before neointima formation and were significantly increased in allografts at all time points. Tumor necrosis factor-alpha and interleukin-1beta were also significantly increased in adventitia in allografts.

CONCLUSIONS: These results demonstrated that adventitial fibroblasts are activated and can produce cytokines and chemokines before the neointimal hyperplasia. They may exert a potential effect on the development of neointimal hyperplasia in TV.

6. Arsenite induces apoptosis in human mesenchymal stem cells by altering Bcl-2 family proteins and by activating intrinsic pathway.

Toxicol Appl Pharmacol. 2010 May 1;244(3):263-72. Epub 2010 Jan 18.

Yadav S, Shi Y, Wang F, Wang H.

PURPOSE: Environmental exposure to arsenic is an important public health issue. The effects of arsenic on different tissues and organs have been intensively studied. However, the effects of arsenic on bone marrow mesenchymal stem cells (MSCs) have not been reported. This study is designed to investigate the cell death process caused by arsenite and its related underlying mechanisms on MSCs. The rationale is that absorbed arsenic in the blood circulation can reach to the bone marrow and may affect the cell survival of MSCs.

METHODS: MSCs of passage 1 were purchased from Tulane University, grown till 70% confluency level and plated according to the experimental requirements followed by treatment with arsenite at various concentrations and time points. Arsenite (iAs(III)) induced cytotoxic effects were confirmed by cell viability and cell cycle analysis. For the presence of canonic apoptosis markers; DNA damage, exposure of intramembrane phosphotidylserine, protein and m-RNA expression levels were analyzed.

RESULTS: iAs(III) induced growth inhibition, G2-M arrest and apoptotic cell death in MSCs, the apoptosis induced by iAs(III) in the cultured MSCs was, via altering Bcl-2 family proteins and by involving intrinsic pathway.

CONCLUSION: iAs(III) can induce apoptosis in bone marrow-derived MSCs via Bcl-2 family proteins, regulating intrinsic apoptotic pathway. Due to the multipotency of MSC, acting as progenitor cells for a variety of connective tissues including bone, adipose, cartilage and muscle, these effects of arsenic may be important in assessing the health risk of the arsenic compounds and understanding the mechanisms of arsenic-induced harmful effects.

7. Cinnamaldehyde impairs high glucose-induced hypertrophy in renal interstitial fibroblasts.

Toxicol Appl Pharmacol. 2010 Apr 15;244(2):174-80. Epub 2010 Jan 6.

Chao LK, Chang WT, Shih YW, Huang JS.

Cinnamaldehyde is a major and a bioactive compound isolated from the leaves of Cinnamomum osmophloeum kaneh. To explore whether cinnamaldehyde was linked to altered high glucose (HG)-mediated renal tubulointerstitial fibrosis in diabetic nephropathy (DN), the molecular mechanisms of cinnamaldehyde responsible for inhibition of HG-induced hypertrophy in renal interstitial fibroblasts were examined. We found that cinnamaldehyde caused inhibition of HG-induced cellular mitogenesis rather than cell death by either necrosis or apoptosis. There were no changes in caspase 3 activity, cleaved poly(ADP-ribose) polymerase (PARP) protein expression, and mitochondrial cytochrome c release in HG or cinnamaldehyde treatments in these cells. HG-induced extracellular signal-regulated kinase (ERK)/c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinase (MAPK) (but not the Janus kinase 2/signal transducers and activators of transcription) activation was markedly blocked by cinnamaldehyde. The ability of cinnamaldehyde to inhibit HG-induced hypertrophy was verified by the observation that it significantly decreased cell size, cellular hypertrophy index, and protein levels of collagen IV, fibronectin, and alpha-smooth muscle actin (alpha-SMA). The results obtained in this study suggest that cinnamaldehyde treatment of renal interstitial fibroblasts that have been stimulated by HG reduces their ability to proliferate and hypertrophy through mechanisms that may be dependent on inactivation of the ERK/JNK/p38 MAPK pathway.

8. Myoblast fusion: when it takes more to make one.

Dev Biol. 2010 May 1;341(1):66-83. Epub 2009 Nov 20.

Rochlin K, Yu S, Roy S, Baylies MK.

Cell-cell fusion is a crucial and highly regulated event in the genesis of both form and function of many tissues. One particular type of cell fusion, myoblast fusion, is a key cellular process that shapes the formation and repair of muscle. Despite its importance for human health, the mechanisms underlying this process are still not well understood. The purpose of this review is to highlight the recent literature pertaining to myoblast fusion and to focus on a comparison of these studies across several model systems, particularly the fly, zebrafish and mouse. Advances in technical analysis and imaging have allowed identification of new fusion genes and propelled further characterization of previously identified genes in each of these systems. Among the cellular steps identified as critical for myoblast fusion are migration, recognition, adhesion, membrane alignment and membrane pore formation and resolution. Importantly, striking new evidence indicates that orthologous genes govern several of these steps across these species. Taken together, comparisons across three model systems are illuminating a once elusive process, providing exciting new insights and a useful framework of genes and mechanisms.

9. Transcription factor GATA-6 is expressed in quiescent myofibroblasts in idiopathic pulmonary fibrosis.

Am J Respir Cell Mol Biol. 2010 May;42(5):626-32. Epub 2009 Jul 13.

Leppäranta O, Pulkkinen V, Koli K, Vähätalo R, Salmenkivi K, Kinnula VL, Heikinheimo M, Myllärniemi M.

Idiopathic pulmonary fibrosis (IPF) (histopathology of usual interstitial pneumonia [UIP]) is a progressive disease with poor prognosis. Characteristic features of IPF/UIP include fibroblastic foci, which are patchy lesions of focal, disarranged myofibroblasts. GATA-6 is a transcription factor linked with cell differentiation. Its role in the development of IPF has not previously been investigated. We hypothesized that GATA-6 participates in the

differentiation of fibroblasts into myofibroblasts in IPF/UIP lungs. The expression patterns of GATA-6, the mesenchymal marker alpha-smooth muscle actin (alpha-SMA), and markers for proliferation (Ki67) and apoptosis (caspase-3) were analyzed in human IPF/UIP tissue samples. The effects of GATA-6 overexpression and silencing were studied in cell cultures. The results show that the alpha-SMA-positive fibroblastic foci in IPF/UIP lungs are positive for GATA-6, but negative for Ki67 and caspase-3. Cultured human IPF/UIP fibroblasts expressed GATA-6 mRNA, whereas cells from the normal adult lung did not. In cultured A549 lung epithelial cells, the induction of GATA-6 by transforming growth factor-beta1 resulted in simultaneous expression of alpha-SMA and decrease of E-cadherin. The inhibition of GATA-6 expression in fibroblasts showed that GATA-6 mediates the alpha-SMA-inducing signal of transforming growth factor-beta1. In conclusion, the hallmark of IPF/UIP histopathology, the fibroblast focus, consists of differentiated, quiescent cells that prominently express GATA-6.

10. Conditional overexpression of connective tissue growth factor disrupts postnatal lung development.

Am J Respir Cell Mol Biol. 2010 May;42(5):552-63. Epub 2009 Jun 18.

Wu S, Platteau A, Chen S, McNamara G, Whitsett J, Bancalari E.

Connective tissue growth factor (CTGF) is a member of an emerging family of immediate-early gene products that coordinates complex biological processes during development, differentiation, and tissue repair. Overexpression of CTGF is associated with mechanical ventilation with high tidal volume and oxygen exposure in newborn lungs. However, the role of CTGF in postnatal lung development and remodeling is not well understood. In the present study, a double-transgenic mouse model was generated with doxycycline-inducible overexpression of CTGF in respiratory epithelial cells. Overexpression of CTGF from Postnatal Days 1-14 resulted in thicker alveolar septa and decreased secondary septal formation. This is correlated with increased myofibroblast differentiation and disorganized elastic fiber deposition in alveolar septa. Overexpression of CTGF also decreased alveolar capillary network formation. There were increased alpha-smooth muscle actin expression and collagen deposition, and dramatic thickening in the peribronchial/peribronchiolar and perivascular regions in the double-transgenic lungs. Furthermore, overexpression of CTGF increased integrin-linked kinase expression, activated its downstream signaling target, Akt, as well as increased mRNA expression of fibronectin. These data demonstrate that overexpression of CTGF disrupts alveologenesis and capillary formation, and induces fibrosis during the critical period of alveolar development. These histologic changes are similar to those observed in lungs of infants with bronchopulmonary dysplasia.

11. Skin-derived precursor cells enhance peripheral nerve regeneration following chronic denervation.

Exp Neurol. 2010 May;223(1):221-8. Epub 2009 May 27.

Walsh SK, Gordon T, Addas BM, Kemp SW, Midha R.

While peripheral nerves demonstrate the capacity for axonal regeneration, outcome following injury remains relatively poor, especially following prolonged denervation. Since axon-deprived Schwann cells (SCs) in the distal nerve progressively lose their ability to support axonal growth, we took the approach of using skin-derived precursor cells (SKPs) as an accessible source of replacement SCs that could be transplanted into chronically denervated peripheral nerve. In this study, we employed a delayed cross-reinnervation paradigm to assess regeneration of common peroneal nerve axons into the chronically denervated rodent tibial nerve following delivery of SKP-derived SC (SKP-SCs). SKP-SC treated animals exhibited superior axonal regeneration to media controls, with significantly higher counts of regenerated motorneurons and histological recovery similar to that of immediately repaired nerve. Improved axonal regeneration correlated with superior muscle reinnervation, as measured by compound muscle action potentials and wet muscle weights. We therefore conclude that SKPs represent an easily accessible, autologous source of stem cell-derived Schwann cells that show promise in improving regeneration through chronically injured nerves.

STEM CELLS AND EPITHELIA

1. Immune promotion of epithelial-mesenchymal transition and generation of breast cancer stem cells.

Cancer Res. 2010 Apr 15;70(8):3005-8.

Reiman JM, Knutson KL, Radisky DC.

Elements of the immune system act as intimate regulators of cancer progression, inhibiting early stages of tumor growth, through immunosurveillance while facilitating later stages of tumor progression. Recent findings have revealed that activated CD8 T cells can stimulate mammary epithelial tumor cells to undergo epithelial-mesenchymal transition (EMT) and to acquire the greatly increased tumorigenic capability and chemotherapeutic resistance of breast cancer stem cells (BCSC). These studies provide a window to understanding how BCSC arise and are maintained within tumors, and how to best target these processes for therapeutic benefit.

2. Induction of cancer cell death by self-assembling nanostructures incorporating a cytotoxic peptide.

Cancer Res. 2010 Apr 15;70(8):3020-6. Epub 2010 Mar 30.

Standley SM, Toft DJ, Cheng H, Soukasene S, Chen J, Raja SM, Band V, Band H, Cryns VL, Stupp SI.

Nanotechnology offers novel delivery vehicles for cancer therapeutics. Potential advantages of nanoscale platforms include improved pharmacokinetics, encapsulation of cytotoxic agents, enhanced accumulation of therapeutics in the tumor microenvironment, and improved therapeutic structures and bioactivity. Here, we report the design of a novel amphiphilic molecule that self-assembles into nanostructures for intracellular delivery of cytotoxic peptides. Specifically, a cationic alpha-helical (KLAKLAK)(2) peptide that is known to induce cancer cell death by membrane disruption was integrated into a peptide amphiphile (PA) that self-assembles into bioactive, cylindrical nanofibers. PAs are composed of a hydrophobic alkyl tail, a beta-sheet forming peptide, and a bioactive peptide that is displayed on the surface of the nanofiber after self-assembly. PA nanostructures that included (KLAKLAK)(2) were readily internalized by breast cancer cells, in contrast to the (KLAKLAK)(2) peptide that on its own was not cell permeable. (KLAKLAK)(2) nanostructures, but not the peptides alone, also induced breast cancer cell death by caspase-independent and Bax/Bak-independent mechanisms associated with membrane disruption. Significantly, (KLAKLAK)(2) nanostructures induced cell death more robustly in transformed breast epithelial cells than in untransformed cells, suggesting a degree of tumor selectivity. Our results provide proof-of-principle that self-assembling PAs can be rationally designed to generate nanostructures that can efficiently deliver cytotoxic peptides to cancer cells. (c) 2010 AACR.

3. Abnormal hair follicle development and altered cell fate of follicular keratinocytes in transgenic mice expressing DeltaNp63alpha.

Development. 2010 May;137(9):1431-9. Epub 2010 Mar 24.

Romano RA, Smalley K, Liu S, Sinha S.

The transcription factor p63 plays an essential role in epidermal morphogenesis. Animals lacking p63 fail to form many ectodermal organs, including the skin and hair follicles. Although the indispensable role of p63 in stratified epithelial skin development is well established, relatively little is known about this transcriptional regulator in directing hair follicle morphogenesis. Here, using specific antibodies, we have established the expression pattern of DeltaNp63 in hair follicle development and cycling. DeltaNp63 is expressed in the developing hair placode, whereas in mature hair its expression is restricted to the outer root sheath (ORS), matrix cells and to the stem cells of the hair follicle bulge. To investigate the role of DeltaNp63 in hair follicle morphogenesis and cycling, we have utilized a Tet-inducible mouse model system with targeted expression of this isoform to the ORS of the hair follicle. DeltaNp63 transgenic animals display dramatic defects in hair follicle development and cycling, eventually leading to severe hair loss. Strikingly, expression of DeltaNp63 leads to a switch in cell fate of hair follicle keratinocytes, causing them to adopt an interfollicular epidermal (IFE) cell identity. Moreover, DeltaNp63 transgenic animals exhibit a depleted hair follicle stem-cell niche, which further contributes to the

overall cycling defects observed in the mutant animals. Finally, global transcriptome analysis of transgenic skin identified altered expression levels of crucial mediators of hair morphogenesis, including key members of the Wnt/beta-catenin signaling pathway, which, in part, account for these effects. Our data provide evidence supporting a role for DeltaNp63alpha in actively suppressing hair follicle differentiation and directing IFE cell lineage commitment.

4. Expansion of CD133(+) colon cancer cultures retaining stem cell properties to enable cancer stem cell target discovery.

Br J Cancer. 2010 Apr 13;102(8):1265-75. Epub 2010 Mar 23.

Fang DD, Kim YJ, Lee CN, Aggarwal S, McKinnon K, Mesmer D, Norton J, Birse CE, He T, Ruben SM, Moore PA.

BACKGROUND: Despite earlier studies demonstrating in vitro propagation of solid tumour cancer stem cells (CSCs) as non-adherent tumour spheres, it remains controversial as to whether CSCs can be maintained in vitro. Additional validation of the CSC properties of tumour spheres would support their use as CSC models and provide an opportunity to discover additional CSC cell surface markers to aid in CSC detection and potential elimination.

METHODS: Primary tumour cells isolated from 13 surgically resected colon tumour specimens were propagated using serum-free CSC-selective conditions. The CSC properties of long-term cultured tumour spheres were established and mass spectrometry-based proteomics performed.

RESULTS: Freshly isolated CD133(+) colorectal cancer cells gave rise to long-term tumour sphere (or spheroids) cultures maintaining CD133 expression. These spheroid cells were able to self-renew and differentiate into adherent epithelial lineages and recapitulate the phenotype of the original tumour. Relative to their differentiated progeny, tumour spheroid cells were more resistant to the chemotherapeutic irinotecan. Finally, CD44, CD166, CD29, CEACAM5, cadherin 17, and biglycan were identified by mass spectrometry to be enriched in CD133(+) tumour spheroid cells.

CONCLUSION: Our data suggest that ex vivo-expanded colon CSCs isolated from clinical specimens can be maintained in culture enabling the identification of CSC cell surface-associated proteins.

5. Differentiation of mouse embryonic stem cells into dental epithelial-like cells induced by ameloblasts serum-free conditioned medium.

Biochem Biophys Res Commun. 2010 Apr 2;394(2):342-7. Epub 2010 Mar 4.

Ning F, Guo Y, Tang J, Zhou J, Zhang H, Lu W, Gao Y, Wang L, Pei D, Duan Y, Jin Y.

Embryonic stem cells (ESCs) possess an intrinsic self-renewal ability and can differentiate into numerous types of functional tissue cells; however, whether ESCs can differentiate toward the odontogenic lineage is still unknown. In this study, we developed an efficient culture strategy to induce the differentiation of murine ESCs (mESCs) into dental epithelial cells. By culturing mESCs in ameloblasts serum-free conditioned medium (ASF-CM), we could induce their differentiation toward dental epithelial cell lineages; however, similar experiments with the tooth germ cell-conditioned medium (TGC-CM) did not yield effective results. After culturing the cells for 14days in the differentiation-inducing media, the expression of ameloblast-specific proteins such as cytokeratin (CK)14, ameloblastin (AMBN), and amelogenin (AMGN) was markedly higher in mESCs obtained with embryoid body (EB) formation than in mESCs obtained without EB formation. We observed that immunocompromised mice implanted with induced murine EBs (mEBs) showed tissue regenerative capacity and produced odontogenic epithelial-like structures, whereas those implanted with mSCE monolayer cells mainly formed connective tissues. Thus, for the first time, we report that ASF-CM provides a suitable microenvironment for inducing mESC differentiation along the odontogenic epithelial cell lineage. This result has important implications for tooth tissue engineering.

6. Transplantation of reprogrammed embryonic stem cells improves visual function in a mouse model for retinitis pigmentosa.

Transplantation. 2010 Apr 27;89(8):911-9.

Wang NK, Tosi J, Kasanuki JM, Chou CL, Kong J, Parmalee N, Wert KJ, Allikmets R, Lai CC, Chien CL, Nagasaki T, Lin CS, Tsang SH.

BACKGROUND: To study whether C57BL/6J-Tyr/J (C2J) mouse embryonic stem (ES) cells can differentiate into retinal pigment epithelial (RPE) cells in vitro and then restore retinal function in a model for retinitis pigmentosa: Rpe65/Rpe65 C57BL6 mice.

METHODS: Yellow fluorescent protein (YFP)-labeled C2J ES cells were induced to differentiate into RPE-like structures on PA6 feeders. RPE-specific markers are expressed from differentiated cells in vitro. After differentiation, ES cell-derived RPE-like cells were transplanted into the subretinal space of postnatal day 5 Rpe65/Rpe65 mice. Live imaging of YFP-labeled C2J ES cells demonstrated survival of the graft. Electroretinograms (ERGs) were performed on transplanted mice to evaluate the functional outcome of transplantation.

RESULTS: RPE-like cells derived from ES cells sequentially express multiple RPE-specific markers. After transplantation, YFP-labeled cells can be tracked with live imaging for as long as 7 months. Although more than half of the mice were complicated with retinal detachments or tumor development, one fourth of the mice showed increased electroretinogram responses in the transplanted eyes. Rpe65/Rpe65 mice transplanted with RPE-like cells showed significant visual recovery during a 7-month period, whereas those injected with saline, PA6 feeders, or undifferentiated ES cells showed no rescue.

CONCLUSIONS: ES cells can differentiate, morphologically, and functionally, into RPE-like cells. Based on these findings, differentiated ES cells have the potential for the development of new therapeutic approaches for RPE-specific diseases such as certain forms of retinitis pigmentosa and macular degeneration. Nevertheless, stringent control of retinal detachment and teratoma development will be necessary before initiation of treatment trials.

7. The Müllerian HOXA10 gene promotes growth of ovarian surface epithelial cells by stimulating epithelial-stromal interactions.

Mol Cell Endocrinol. 2010 Apr 12;317(1-2):112-9. Epub 2009 Dec 29. PMID: 20036708 [PubMed - indexed for MEDLINE]PMCID: PMC2814902 [Available on 2011/4/12]

Ko SY, Lengyel E, Naora H.

The ovarian surface epithelium (OSE) origin of ovarian cancers has been controversial because these cancers often exhibit Müllerian-like features. One hypothesis is that ovarian neoplasia involves the gain of growth advantages by OSE cells via activation of Müllerian programs. The homeobox gene HOXA10 controls formation of the uterus from the Müllerian ducts, and is not expressed in normal OSE. We previously found that HOXA10 is expressed in ovarian cancers with endometrial-like features, and induces transformed OSE cells to form glandular tumors in mice. In the current study, we found that induction of HOXA10 in OSE cells promotes homophilic cell adhesion and prevents anoikis. HOXA10 expression stimulated interactions of OSE cells with the extracellular matrix proteins vitronectin and fibronectin, and with mesothelial cells of the omentum which is a common attachment site for ovarian cancer cells. HOXA10 also stimulated interactions of OSE cells with omental fibroblasts, and these interactions promoted OSE cell growth. Our findings indicate that aberrant activation of a Müllerian program in OSE cells confers growth advantages by stimulating cellular interactions with the microenvironment.

8. The developmental expression profile of PAX2 in the murine prostate.

Prostate. 2010 May 1;70(6):654-65.

Chen Q, DeGraff DJ, Sikes RA.

BACKGROUND: Nine transcription factors comprise the PAX gene family that regulate organogenesis. The urogenital system of PAX2 null male mice fails to develop properly. PAX2 is overexpressed in PC3 cells. Therefore, PAX2 is implicated in both prostate organogenesis and cancer. However, the expression pattern/profile of PAX2 in the prostate is unknown.

METHODS: PAX2/5/8 expression was surveyed in E16.5 male urogenital sinus (UGS) by RT-PCR. Prostate samples from 10 developmental stages in C3H male mice were used in quantitative reverse-transcript PCR (Q-PCR) and Western blotting (WB). RT-PCR and WB measured PAX2 expression in prostatic lobes or UGS layers, to identify local-regional expression patterns. Cytoplasmic versus nuclear expression was examined by WB. A castration series in adult C3H male mice and R1881 treatment in serum-free LNCaP cells examined androgen control of PAX2.

RESULTS: PAX2 mRNA levels are higher in early developmental stages as compared to postpubertal prostates. RT-PCR and/or WB indicated a dorsal epithelial-nuclear localization of PAX2. PAX2 mRNA and protein increase postcastration. R1881 decreases expression of PAX2 mRNA in LNCaP cells as compared to controls.

CONCLUSIONS: The expression profile of PAX2 indicates that it may regulate early, androgen-independent stages of murine prostate development, particularly for dorsally derived prostate glands. PAX2 expression appears to be associated with a dorsally localized epithelial cell population that is castration insensitive and retains proliferative and differentiative potential. Such a population of cells may represent a subset of stem-like cells having some characteristics in common with castrate-resistant prostate cancer cells.

9. Transcription factor GATA-6 is expressed in quiescent myofibroblasts in idiopathic pulmonary fibrosis.

Am J Respir Cell Mol Biol. 2010 May;42(5):626-32. Epub 2009 Jul 13.

Leppäranta O, Pulkkinen V, Koli K, Vähätalo R, Salmenkivi K, Kinnula VL, Heikinheimo M, Myllärniemi M.

Idiopathic pulmonary fibrosis (IPF) (histopathology of usual interstitial pneumonia [UIP]) is a progressive disease with poor prognosis. Characteristic features of IPF/UIP include fibroblastic foci, which are patchy lesions of focal, disarranged myofibroblasts. GATA-6 is a transcription factor linked with cell differentiation. Its role in the development of IPF has not previously been investigated. We hypothesized that GATA-6 participates in the differentiation of fibroblasts into myofibroblasts in IPF/UIP lungs. The expression patterns of GATA-6, the mesenchymal marker alpha-smooth muscle actin (alpha-SMA), and markers for proliferation (Ki67) and apoptosis (caspase-3) were analyzed in human IPF/UIP tissue samples. The effects of GATA-6 overexpression and silencing were studied in cell cultures. The results show that the alpha-SMA-positive fibroblastic foci in IPF/UIP lungs are positive for GATA-6, but negative for Ki67 and caspase-3. Cultured human IPF/UIP fibroblasts expressed GATA-6 mRNA, whereas cells from the normal adult lung did not. In cultured A549 lung epithelial cells, the induction of GATA-6 by transforming growth factor-beta1 resulted in simultaneous expression of alpha-SMA and decrease of E-cadherin. The inhibition of GATA-6 expression in fibroblasts showed that GATA-6 mediates the alpha-SMA-inducing signal of transforming growth factor-beta1. In conclusion, the hallmark of IPF/UIP histopathology, the fibroblast focus, consists of differentiated, quiescent cells that prominently express GATA-6.

10. Conditional overexpression of connective tissue growth factor disrupts postnatal lung development.

Am J Respir Cell Mol Biol. 2010 May;42(5):552-63. Epub 2009 Jun 18.

Wu S, Platteau A, Chen S, McNamara G, Whitsett J, Bancalari E.

Connective tissue growth factor (CTGF) is a member of an emerging family of immediate-early gene products that coordinates complex biological processes during development, differentiation, and tissue repair. Overexpression of CTGF is associated with mechanical ventilation with high tidal volume and oxygen exposure in newborn lungs. However, the role of CTGF in postnatal lung development and remodeling is not well understood. In the present study, a double-transgenic mouse model was generated with doxycycline-inducible overexpression of CTGF in respiratory epithelial cells. Overexpression of CTGF from Postnatal Days 1-14 resulted in thicker alveolar septa and decreased secondary septal formation. This is correlated with increased

myofibroblast differentiation and disorganized elastic fiber deposition in alveolar septa. Overexpression of CTGF also decreased alveolar capillary network formation. There were increased alpha-smooth muscle actin expression and collagen deposition, and dramatic thickening in the peribronchial/peribronchiolar and perivascular regions in the double-transgenic lungs. Furthermore, overexpression of CTGF increased integrin-linked kinase expression, activated its downstream signaling target, Akt, as well as increased mRNA expression of fibronectin. These data demonstrate that overexpression of CTGF disrupts alveologenesis and capillary formation, and induces fibrosis during the critical period of alveolar development. These histologic changes are similar to those observed in lungs of infants with bronchopulmonary dysplasia.

MUSCLE CELL BIOLOGY

1. The FoxF/FoxC factor LET-381 directly regulates both cell fate specification and cell differentiation in C. elegans mesoderm development.

Development. 2010 May;137(9):1451-60. Epub 2010 Mar 24.

Amin NM, Shi H, Liu J.

Forkhead transcription factors play crucial and diverse roles in mesoderm development. In particular, FoxF and FoxC genes are, respectively, involved in the development of visceral/splanchnic mesoderm and non-visceral mesoderm in coelomate animals. Here, we show at single-cell resolution that, in the pseudocoelomate nematode C. elegans, the single FoxF/FoxC transcription factor LET-381 functions in a feed-forward mechanism in the specification and differentiation of the non-muscle mesodermal cells, the coelomocytes (CCs). LET-381/FoxF directly activates the CC specification factor, the Six2 homeodomain protein CEH-34, and functions cooperatively with CEH-34/Six2 to directly activate genes required for CC differentiation. Our results unify a diverse set of studies on the functions of FoxF/FoxC factors and provide a model for how FoxF/FoxC factors function during mesoderm development.

2. TGF-beta mediated FGF10 signaling in cranial neural crest cells controls development of myogenic progenitor cells through tissue-tissue interactions during tongue morphogenesis.

Dev Biol. 2010 May 1;341(1):186-95. Epub 2010 Feb 26.

Hosokawa R, Oka K, Yamaza T, Iwata J, Urata M, Xu X, Bringas P Jr, Nonaka K, Chai Y.

Skeletal muscles are formed from two cell lineages, myogenic and fibroblastic. Mesoderm-derived myogenic progenitors form muscle cells whereas fibroblastic cells give rise to the supportive connective tissue of skeletal muscles, such as the tendons and perimysium. It remains unknown how myogenic and fibroblastic cell-cell interactions affect cell fate determination and the organization of skeletal muscle. In the present study, we investigated the functional significance of cell-cell interactions in regulating skeletal muscle development. Our study shows that cranial neural crest (CNC) cells give rise to the fibroblastic cells of the tongue skeletal muscle in mice. Loss of Tgfbr2 in CNC cells (Wnt1-Cre;Tgfbr2(flox/flox)) results in microglossia with reduced Scleraxis and Fgf10 expression as well as decreased myogenic cell proliferation, reduced cell number and disorganized tongue muscles. Furthermore, TGF-beta2 beads induced the expression of Scleraxis in tongue explant cultures. The addition of FGF10 rescued the muscle cell number in Wnt1-Cre;Tgfbr2(flox/flox) mice. Thus, TGF-beta induced FGF10 signaling has a critical function in regulating tissue-tissue interaction during tongue skeletal muscle development.

3. Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes.

Circ Res. 2010 Apr 16;106(7):1253-64. Epub 2010 Feb 25.

Ago T, Kuroda J, Pain J, Fu C, Li H, Sadoshima J.

RATIONALE: NADPH oxidases are a major source of superoxide (O(2)(-)) in the cardiovascular system. The function of Nox4, a member of the Nox family of NADPH oxidases, in the heart is poorly understood.

OBJECTIVE: The goal of this study was to elucidate the role of Nox4 in mediating oxidative stress and growth/death in the heart.

METHODS AND RESULTS: Expression of Nox4 in the heart was increased in response to hypertrophic stimuli and aging. Neither transgenic mice with cardiac specific overexpression of Nox4 (Tg-Nox4) nor those with catalytically inactive Nox4 (Tg-Nox4-P437H) showed an obvious baseline cardiac phenotype at young ages. Tg-Nox4 gradually displayed decreased left ventricular (LV) function with enhanced O(2)(-) production in the heart, which was accompanied by increased apoptosis and fibrosis at 13 to 14 months of age. On the other hand, the level of oxidative stress was attenuated in Tg-Nox4-P437H. Although the size of cardiac myocytes was significantly greater in Tg-Nox4 than in nontransgenic, the LV weight/tibial length was not significantly altered in Tg-Nox4 mice. Overexpression of Nox4 in cultured cardiac myocytes induced apoptotic cell death but not hypertrophy. Nox4 is primarily localized in mitochondria and upregulation of Nox4 enhanced both rotenone- and diphenyleneiodonium-sensitive O(2)(-) production in mitochondria. Cysteine residues in mitochondrial proteins, including aconitase and NADH dehydrogenases, were oxidized and their activities decreased in Tg-Nox4.

CONCLUSIONS: Upregulation of Nox4 by hypertrophic stimuli and aging induces oxidative stress, apoptosis and LV dysfunction, in part because of mitochondrial insufficiency caused by increased O(2)(-) production and consequent cysteine oxidation in mitochondrial proteins.

4. A comparison of the postnatal development of muscle-spindle and periodontal-ligament neurons in the mesencephalic trigeminal nucleus of the rat.

Neurosci Lett. 2010 Apr 5;473(2):155-7. Epub 2010 Feb 23.

Umemura T, Yasuda K, Ishihama K, Yamada H, Okayama M, Hasumi-Nakayama Y, Furusawa K.

The trigeminal mesencephalic nucleus (Vmes) is known to include primary afferent neurons of jaw muscle spindles (MS neurons) and periodontal ligament receptors (PL neurons). The aim of this study was to clarify the postnatal development of Vmes neurons by comparing MS neurons with PL neurons using horseradish peroxidase labeling. We measured somal diameter and somal shape of MS and PL neurons in rats from postnatal day (P)7 to P70. No significant changes were seen between postnatal day P7 and P70 in somal diameter or somal shape of MS neurons. Conversely, PL neurons showed a larger somal diameter at P7 than at P14, and in terms of somal profile, multipolar neurons comprised 0% at P7, but 4.8% at P14 and 16.9% at P70. These findings suggest that PL neurons develop with the eruption of teeth, taking into account the fact that tooth eruption occurs from around P14 in rats. Conversely, the lack of postnatal changes in MS neurons is due to the fact that these neurons have been active since the embryonic period, as swallowing starts in utero.

5. Plasminogen activator inhibitor-1 regulates myoendothelial junction formation.

Circ Res. 2010 Apr 2;106(6):1092-102. Epub 2010 Feb 4. Comment in: Circ Res. 2010 Apr 2;106(6):1014-6.

Heberlein KR, Straub AC, Best AK, Greyson MA, Looft-Wilson RC, Sharma PR, Meher A, Leitinger N, Isakson BE.

RATIONALE: Plasminogen activator inhibitor-1 (PAI-1) is a biomarker for several vascular disease states; however, its target of action within the vessel wall is undefined.

OBJECTIVE: Determine the ability of PAI-1 to regulate myoendothelial junction (MEJ) formation.

METHODS AND RESULTS: MEJs are found throughout the vasculature linking endothelial cells (ECs) and vascular smooth muscle cells. Using a vascular cell coculture we isolated MEJ fractions and performed two-dimensional differential gel electrophoresis. Mass spectrometry identified PAI-1 as being enriched within MEJ fractions, which we confirmed in vivo. In the vascular cell coculture, recombinant PAI-1 added to the EC monolayer significantly increased MEJs. Conversely, addition of a PAI-1 monoclonal antibody to the EC monolayer reduced the number of MEJs. This was also observed in vivo where mice fed a high fat diet had increased PAI-1 and MEJs and the number of MEJs in coronary arterioles of PAI-1(-/-) mice was significantly reduced when compared to C57Bl/6 mice. The presence of MEJs in PAI-1(-/-) coronary arterioles was restored when their hearts were transplanted into and exposed to the circulation of C57Bl/6 mice. Application of biotin-conjugated PAI-1 to the EC monolayer in vitro confirmed the ability of luminal PAI-1 to translocate to the MEJ.

Functionally, phenylephrine-induced heterocellular calcium communication in the vascular cell coculture was temporally enhanced when recombinant PAI-1 was present, and prolonged when PAI-1 was absent.

CONCLUSION: Our data implicate circulating PAI-1 as a key regulator of MEJ formation and a potential target for pharmacological intervention in diseases with vascular abnormalities (eg, diabetes mellitus).

6. Myoblast fusion: when it takes more to make one.

Dev Biol. 2010 May 1;341(1):66-83. Epub 2009 Nov 20.

Rochlin K, Yu S, Roy S, Baylies MK.

Cell-cell fusion is a crucial and highly regulated event in the genesis of both form and function of many tissues. One particular type of cell fusion, myoblast fusion, is a key cellular process that shapes the formation and repair of muscle. Despite its importance for human health, the mechanisms underlying this process are still not well understood. The purpose of this review is to highlight the recent literature pertaining to myoblast fusion and to focus on a comparison of these studies across several model systems, particularly the fly, zebrafish and mouse. Advances in technical analysis and imaging have allowed identification of new fusion genes and propelled further characterization of previously identified genes in each of these systems. Among the cellular steps identified as critical for myoblast fusion are migration, recognition, adhesion, membrane alignment and membrane pore formation and resolution. Importantly, striking new evidence indicates that orthologous genes govern several of these steps across these species. Taken together, comparisons across three model systems are illuminating a once elusive process, providing exciting new insights and a useful framework of genes and mechanisms.

EPITHELIAL CELL BIOLOGY

1. Mammalian target of rapamycin activator RHEB is frequently overexpressed in human carcinomas and is critical and sufficient for skin epithelial carcinogenesis.

Cancer Res. 2010 Apr 15;70(8):3287-98. Epub 2010 Apr 13.

Lu ZH, Shvartsman MB, Lee AY, Shao JM, Murray MM, Kladney RD, Fan D, Krajewski S, Chiang GG, Mills GB, Arbeit JM.

Small GTPase Ras homologue enriched in brain (RHEB) binds and activates the key metabolic regulator mTORC1, which has an important role in cancer cells, but the role of RHEB in cancer pathogenesis has not been shown. By performing a meta-analysis of published cancer cytogenetic and transcriptome databases, we defined a gain of chromosome 7q36.1-q36.3 containing the RHEB locus, an overexpression of RHEB mRNA in several different carcinoma histotypes, and an association between RHEB upregulation and poor prognosis in breast and head and neck cancers. To model gain of function in epithelial malignancy, we targeted Rheb expression to murine basal keratinocytes of transgenic mice at levels similar to those that occur in human squamous cancer cell lines. Juvenile transgenic epidermis displayed constitutive mTORC1 pathway activation, elevated cyclin D1 protein, and diffuse skin hyperplasia. Skin tumors subsequently developed with concomitant stromal angio-inflammatory foci, evidencing induction of an epidermal hypoxia-inducible factor-1 transcriptional program, and paracrine feed-forward activation of the interleukin-6-signal transducer and activator of transcription 3 pathway. Rheb-induced tumor persistence and neoplastic molecular alterations were mTORC1 dependent. Rheb markedly sensitized transgenic epidermis to squamous carcinoma induction following a single dose of Ras-activating carcinogen 7,12-dimethylbenz(a)anthracene. Our findings offer direct evidence that RHEB facilitates multistage carcinogenesis through induction of multiple oncogenic mechanisms, perhaps contributing to the poor prognosis of patients with cancers overexpressing RHEB.

2. Analysis of chemotherapy response programs in ovarian cancers by the next-generation sequencing technologies.

Gynecol Oncol. 2010 May;117(2):159-69. Epub 2010 Feb 23.

Cheng L, Lu W, Kulkarni B, Pejovic T, Yan X, Chiang JH, Hood L, Odunsi K, Lin B.

OBJECTIVE: To understand the chemotherapy response program in ovarian cancer cells at deep transcript sequencing levels. METHODS: Two next-generation sequencing technologies--MPSS (massively parallel signature sequencing) and SBS (sequencing by synthesis)--were used to sequence the transcripts of IGROV1 and IGROV1-CP cells, and to sequence the transcripts of a highly chemotherapy responsive and a highly chemotherapy resistant ovarian cancer tissue.

RESULTS: We identified 3422 signatures (2957 genes) that are significantly different between IGROV1 and IGROV1-CP cells (P<0.001). Gene Ontology (GO) term GO:0001837 (epithelial-to-mesenchymal transition) and GO:0034330 (cell junction assembly and maintenance) are enriched in genes that are over expressed in IGROV1-CP cells while apoptosis-related GO terms are enriched in genes over expressed in IGROV1 cells. We identified 1187 tags (corresponding to 1040 genes) that are differentially expressed between the chemotherapy responsive and the persistently chemotherapy resistant ovarian cancer tissues. GO term GO:0050673 (epithelial cell proliferation) and GO:0050678 (regulation of epithelial cell proliferation) are enriched in the genes over expressed in the chemotherapy resistant tissue while the GO:0007229 (integrin-mediated signaling pathway) is enriched in the genes over expressed in the chemotherapy sensitive tissue. An integrative analysis identified 111 common differentially expressed genes including two bone morphogenetic proteins (BMP4 and BMP7), six solute carrier proteins (SLC10A3, SLC16A3, SLC25A1, SLC35B3, SLC7A5 and SLC7A7), transcription factor POU5F1 (POU class 5 homeobox 1), and KLK10 (kallikrein-related peptidase 10). A network analysis revealed a subnetwork with three genes BMP7, NR2F2 and AP2B1 that were consistently over expressed in the chemoresistant tissue or cells compared to the chemosensitive tissue or cells.

CONCLUSION: Our database offers the first comprehensive view of the digital transcriptomes of ovarian cancer cell lines and tissues with different chemotherapy response phenotypes.

3. Inhibition of aldose reductase attenuates endotoxin signals in human non-pigmented ciliary epithelial cells.

Exp Eye Res. 2010 May;90(5):555-63. Epub 2010 Feb 4.

Yadav UC, Srivastava SK, Ramana KV.

Chronic inflammatory diseases such as autoimmune and bacterial infections are associated with an elevated risk of ocular inflammation. Ciliary epithelial cells that play an important role in maintaining aqueous humor dynamics and homeostasis of anterior segment of eye are continuously exposed to inflammatory markers during infections and injury. Lipopolysacchharide (LPS), a Gram-negative bacterial endotoxin, dysregulates aqueous humor (AqH) homeostasis by inducing inflammatory changes. We have investigated how inhibition of a polyol pathway enzyme, aldose reductase (AR), alters LPS-induced inflammatory changes in human non-pigmented ciliary epithelial cells (hNPECs). The stimulation of hNPECs with LPS (1 microg/ml) caused increased secretion of inflammatory markers such as PGE(2) and NO in the culture medium as well as increased expression of COX-2 and iNOS proteins in cell extracts. LPS also increased phosphorylation of MAPKs (ERK1/2) and SAPK/JNK and activation of redox-sensitive transcription factors NF-kappaB and AP-1 in hNPECs and inhibition of AR by zopolrestat and sorbinil ameliorated these changes. Further, LPS-induced decrease in the expression of Na/K-ATPase in hNPECs was restored by AR inhibitors. Similar results were observed in ciliary bodies of LPS-injected rats. Taken together, our results suggest that AR plays an important role in the LPS-induced inflammatory changes in hNPECs and that inhibition of AR could be a novel therapeutic approach for ocular inflammation.

4. DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells.

Environ Mol Mutagen. 2010 May;51(4):304-14.

Attene-Ramos MS, Nava GM, Muellner MG, Wagner ED, Plewa MJ, Gaskins HR.

Hydrogen sulfide (H(2)S), a metabolic end product of sulfate-reducing bacteria, represents a genotoxic insult to the colonic epithelium, which may also be linked with chronic disorders such as ulcerative colitis and colorectal cancer. This study defined the early (30 min) and late (4 hr) response of nontransformed human intestinal

epithelial cells (FHs 74 Int) to H(2)S. The genotoxicity of H(2)S was measured using the single-cell gel electrophoresis (comet) assay. Changes in gene expression were analyzed after exposure to a genotoxic, but not cytotoxic, concentration of H(2)S (500 muM H(2)S) using pathway-specific quantitative RT-PCR gene arrays. H(2)S was genotoxic in a concentration range from 250 to 2,000 microM, which is similar to concentrations found in the large intestine. Significant changes in gene expression were predominantly observed at 4 hr, with the greatest responses by PTGS2 (COX-2; 7.92-fold upregulated) and WNT2 (7.08-fold downregulated). COX-2 was the only gene upregulated at both 30 min and 4 hr. Overall, the study demonstrates that H(2)S modulates the expression of genes involved in cell-cycle progression and triggers both inflammatory and DNA repair responses. This study confirms the genotoxic properties of H(2)S in nontransformed human intestinal epithelial cells and identifies functional pathways by which this bacterial metabolite may perturb cellular homeostasis and contribute to the onset of chronic intestinal disorders.

5. The Müllerian HOXA10 gene promotes growth of ovarian surface epithelial cells by stimulating epithelial-stromal interactions.

Mol Cell Endocrinol. 2010 Apr 12;317(1-2):112-9. Epub 2009 Dec 29.

Ko SY, Lengyel E, Naora H.

The ovarian surface epithelium (OSE) origin of ovarian cancers has been controversial because these cancers often exhibit Müllerian-like features. One hypothesis is that ovarian neoplasia involves the gain of growth advantages by OSE cells via activation of Müllerian programs. The homeobox gene HOXA10 controls formation of the uterus from the Müllerian ducts, and is not expressed in normal OSE. We previously found that HOXA10 is expressed in ovarian cancers with endometrial-like features, and induces transformed OSE cells to form glandular tumors in mice. In the current study, we found that induction of HOXA10 in OSE cells promotes homophilic cell adhesion and prevents anoikis. HOXA10 expression stimulated interactions of OSE cells with the extracellular matrix proteins vitronectin and fibronectin, and with mesothelial cells of the omentum which is a common attachment site for ovarian cancer cells. HOXA10 also stimulated interactions of OSE cells with omental fibroblasts, and these interactions promoted OSE cell growth. Our findings indicate that aberrant activation of a Müllerian program in OSE cells confers growth advantages by stimulating cellular interactions with the microenvironment.

6. Cortisol differentially alters claudin isoforms in cultured puffer fish gill epithelia.

Mol Cell Endocrinol. 2010 Apr 12;317(1-2):120-6. Epub 2009 Dec 5.

Bui P, Bagherie-Lachidan M, Kelly SP.

A primary cultured gill epithelium from the puffer fish Tetraodon nigroviridis was developed to examine the corticosteroid regulation of claudin isoform mRNA abundance in fish gills. Preparations were composed of polygonal epithelial cells exhibiting concentric apical microridges and zonula occludens-1 immunoreactivity along cell margins. No evidence was found to indicate the presence of Na(+)-K(+)-ATPase-immunoreactive or mitochondria-rich cells in cultured preparations. Therefore, epithelia appear to be composed of gill pavement cells (PVCs) only. An RT-PCR profile of 12 salinity responsive gill claudin tight junction (TJ) proteins (Tncldn3a, -3c, -6, -8d, -10d, -10e, -11a, -23b, -27a, -27c, -32a, and -33b) revealed the absence of Tncldn6, -10d and -10e in cultured epithelia, suggesting that these isoforms are not associated with gill PVCs. Cortisol treatment of cultured epithelia dose-dependently increased or decreased mRNA abundance of select claudin isoforms. Transcript abundance of several claudin isoforms was unaffected by cortisol treatment. These data provide evidence for the cell specific distribution of claudins in fish gills and suggest that heterogeneous alterations in the abundance of select claudin isoforms contribute to the corticosteroid regulation of gill permeability.

7. Morphogenesis of epithelial tubes: Insights into tube formation, elongation, and elaboration.

Dev Biol. 2010 May 1;341(1):34-55. Epub 2009 Sep 22.

Andrew DJ, Ewald AJ.

Epithelial tubes are a fundamental tissue across the metazoan phyla and provide an essential functional

component of many of the major organs. Recent work in flies and mammals has begun to elucidate the cellular mechanisms driving the formation, elongation, and branching morphogenesis of epithelial tubes during development. Both forward and reverse genetic techniques have begun to identify critical molecular regulators for these processes and have revealed the conserved role of key pathways in regulating the growth and elaboration of tubular networks. In this review, we discuss the developmental programs driving the formation of branched epithelial networks, with specific emphasis on the trachea and salivary gland of Drosophila melanogaster and the mammalian lung, mammary gland, kidney, and salivary gland. We both highlight similarities in the development of these organs and attempt to identify tissue and organism specific strategies. Finally, we briefly consider how our understanding of the regulation of proliferation, apicobasal polarity, and epithelial motility during branching morphogenesis can be applied to understand the pathologic dysregulation of these same processes during metastatic cancer progression.

STEM CELL CLINICAL TRIALS

1. Impact of EWS-ETS fusion type on disease progression in Ewing's sarcoma/peripheral primitive neuroectodermal tumor: prospective results from the cooperative Euro-E.W.I.N.G. 99 trial.

J Clin Oncol. 2010 Apr 20;28(12):1982-8. Epub 2010 Mar 22. Comment in: J Clin Oncol. 2010 Apr 20;28(12):1973-4.

Le Deley MC, Delattre O, Schaefer KL, Burchill SA, Koehler G, Hogendoorn PC, Lion T, Poremba C, Marandet J, Ballet S, Pierron G, Brownhill SC, Nesslböck M, Ranft A, Dirksen U, Oberlin O, Lewis IJ, Craft AW, Jürgens H, Kovar H.

PURPOSE EWS-ETS fusion genes are the driving force in Ewing's sarcoma pathogenesis. Because of the variable breakpoint locations in the involved genes, there is heterogeneity in fusion RNA and protein architecture. Since previous retrospective studies suggested prognostic differences among patients expressing different EWS-FLI1 fusion types, the impact of fusion RNA architecture on disease progression and relapse was studied prospectively within the Euro-E.W.I.N.G. 99 clinical trial.

PATIENTS AND METHODS Among 1,957 patients who registered before January 1, 2007, 703 primary tumors were accessible for the molecular biology study. Fusion type was assessed by polymerase chain reaction on frozen (n = 578) or paraffin-embedded materials (n = 125). The primary end point was the time to disease progression or relapse. Results After exclusion of noninformative patients, 565 patients were entered into the prognostic factor analysis comparing type 1 (n = 296), type 2 (n = 133), nontype 1/nontype 2 EWS-FLI1 (n = 91) and EWS-ERG fusions (n = 45). Median follow-up time was 4.5 years. The distribution of sex, age, tumor volume, tumor site, disease extension, or histologic response did not differ between the four fusion type groups. We did not observe any significant prognostic value of the fusion type on the risk of progression or relapse. The only slight difference was that the risk of progression or relapse associated with nontype 1/nontype 2 EWS-FLI1 fusions was 1.38 (95% CI, 0.96 to 2.0) times higher than risk associated with other fusion types, but it was not significant (P = .10).

CONCLUSION In contrast to retrospective studies, the prospective evaluation did not confirm a prognostic benefit for type 1 EWS-FLI1 fusions.

2. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma.

J Clin Oncol. 2010 Apr 20;28(12):2077-84. Epub 2010 Mar 22.

Ladetto M, Pagliano G, Ferrero S, Cavallo F, Drandi D, Santo L, Crippa C, De Rosa L, Pregno P, Grasso M, Liberati AM, Caravita T, Pisani F, Guglielmelli T, Callea V, Musto P, Cangialosi C, Passera R, Boccadoro M, Palumbo A.

PURPOSE We investigated the effect on minimal residual disease, by qualitative and real-time quantitative polymerase chain reaction (RQ-PCR), of a consolidation regimen that included bortezomib, thalidomide, and dexamethasone (VTD) in patients with multiple myeloma (MM) responding to autologous stem-cell transplantation (auto-SCT).

PATIENTS AND METHODS Patients achieving at least very good partial response who had an available molecular marker based on the immunoglobulin heavy-chain rearrangement received four courses of treatment every month: four infusions per month of bortezomib at 1.6 mg/m(2), thalidomide at 200 mg/d, and dexamethasone at 20 mg/d on days 1 to 4, 8 to 11, and 15 to 18. Patients were studied with tumor-clone-specific primers by qualitative nested PCR and RQ-PCR. Results Of 39 patients enrolled, 31 received the four VTD courses. Immunofixation complete responses increased from 15% after auto-SCT to 49% after VTD. Molecular remissions (MRs) were 3% after auto-SCT and 18% after VTD. Median time to maximum response was 3.5 months. So far, no patient in MR has relapsed (median follow-up, 42 months). VTD consolidation induced an additional depletion of 4.14 natural logarithms of tumor burden by RQ-PCR. Patients with a tumor load less than the median value after VTD had outcomes better than those who had tumor loads above the median value after VTD (at median follow-up: progression-free survival, 100% v 57%; P < .001).

CONCLUSION To the best of our knowledge, this study is the first to document the occurrence of persistent MRs in a proportion of MM patients treated without allogeneic transplantation. Moreover, the major reduction in tumor load recorded by RQ-PCR after VTD suggests that unprecedented levels of tumor cell reduction can be achieved in MM thanks to the new nonchemotherapeutic drugs.

3. Lymphoma recurrence 5 years or later following diffuse large B-cell lymphoma: clinical characteristics and outcome.

J Clin Oncol. 2010 Apr 20;28(12):2094-100. Epub 2010 Mar 22.

Larouche JF, Berger F, Chassagne-Clément C, Ffrench M, Callet-Bauchu E, Sebban C, Ghesquières H, Broussais-Guillaumot F, Salles G, Coiffier B.

PURPOSE Patients with diffuse large B-cell lymphoma (DLBCL) usually relapse early following diagnosis but some relapses happen at 5 years or later. Few data exist regarding clinical characteristics and outcome of these patients. PATIENTS AND METHODS We performed a retrospective analysis of all patients from two centers in Lyon, France, between 1985 and 2003 who had a biopsy-proven relapse 5 years or later following diagnosis of DLBCL. All available biopsies were reviewed and immunohistochemistry was completed. Results Among 1,492 patients with DLBCL, 54 were eligible. At diagnosis, 63% of patients had stage I-II, 82% had low/low-intermediate International Prognostic Index (IPI) score, 65% had extranodal involvement, 24% had an indolent component associated with DLBCL, 57% had germinal center phenotype, and 43% had non-germinal center phenotype. Median time from diagnosis to relapse was 7.4 years (range, 5 to 20.5 years). At time of relapse, 83% had DLBCL histology, and 17% had indolent histology. Having an indolent component at diagnosis was associated with indolent histology at relapse (P = .028). Five-year event free-survival (EFS) was 17% for patients with DLBCL relapse and 61% for patients with indolent relapse (P = .027). Five-year overall survival was 27% for patients with DLBCL and 75% for patients with indolent relapse (P = .029). For DLBCL relapse, 3-year EFS was 56% versus 18% with autologous stem-cell transplantation or not, respectively (P = .0661). CONCLUSION Patients with DLBCL who had a late relapse usually had localized stage, favorable IPI score, and extranodal involvement at diagnosis. The outcome of patients with DLBCL at time of relapse remains poor, and aggressive treatment such as autologous stem-cell transplantation should be pursued whenever possible. Biopsy at relapse is essential because some patients relapse with indolent histology.

4. High EVI1 expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities.

J Clin Oncol. 2010 Apr 20;28(12):2101-7. Epub 2010 Mar 22.

Gröschel S, Lugthart S, Schlenk RF, Valk PJ, Eiwen K, Goudswaard C, van Putten WJ, Kayser S, Verdonck LF, Lübbert M, Ossenkoppele GJ, Germing U, Schmidt-Wolf I, Schlegelberger B, Krauter J, Ganser A, Döhner H, Löwenberg B, Döhner K, Delwel R.

PURPOSE: The purpose of this study was to investigate frequency and prognostic significance of high EVI1 expression in acute myeloid leukemia (AML).

PATIENTS AND METHODS: A diagnostic assay detecting multiple EVI1 splice variants was developed to determine the relative EVI1 expression by single real-time quantitative polymerase chain reaction in 1,382 newly

diagnosed adult patients with AML younger than 60 years. Patients were treated on four Dutch-Belgian HOVON (n = 458) and two German-Austrian AML Study Group protocols (n = 924).

RESULTS; The EVI1 assay was tested in the HOVON cohort and validated in the AMLSG cohort. High EVI1 levels (EVI1(+)) were found with similar frequencies in both cohorts combined, with a 10.7% incidence (148 of 1,382). EVI1(+) independently predicted low complete remission (CR) rate (odds ratio, 0.54; P = .002), adverse relapse-free survival (RFS; hazard ratio [HR], 1.32; P = .05), and event-free survival (EFS; HR, 1.46; P < .001). This adverse prognostic impact was more pronounced in the intermediate cytogenetic risk group (EFS; HR, 1.64; P < .001; and RFS; HR, 1.55; P = .02), and was also apparent in cytogenetically normal AML (EFS; HR, 1.67; P = .008). Besides inv(3)/t(3;3), EVI1(+) was significantly associated with chromosome abnormalities monosomy 7 and t(11q23), conferring prognostic impact within these two cytogenetic subsets. EVI1(+) was virtually absent in favorable-risk AML and AML with NPM1 mutations. Patients with EVI1(+) AML (n = 28) who received allogeneic stem cell transplantation in first CR had significantly better 5-year RFS (33% +/- 10% v 0%).

CONCLUSION: EVI1 expression in AML is unequally distributed in cytogenetic subtypes. It predicts poor outcome, particularly among intermediate cytogenetic risk AML. Patients with EVI1(+) AML may benefit from allogeneic transplantation in first CR. Pretreatment EVI1 screening should be included in risk stratification.

5. Gene expression-based classification as an independent predictor of clinical outcome in juvenile myelomonocytic leukemia.

J Clin Oncol. 2010 Apr 10;28(11):1919-27. Epub 2010 Mar 15.

Bresolin S, Zecca M, Flotho C, Trentin L, Zangrando A, Sainati L, Stary J, de Moerloose B, Hasle H, Niemeyer CM, Te Kronnie G, Locatelli F, Basso G.

PURPOSE Juvenile myelomonocytic leukemia (JMML) is a rare early childhood myelodysplastic/myeloproliferative disorder characterized by an aggressive clinical course. Age and hemoglobin F percentage at diagnosis have been reported to predict both survival and outcome after hematopoietic stem cell transplantation (HSCT). However, no genetic markers with prognostic relevance have been identified so far. We applied gene expression-based classification to JMML samples in order to identify prognostic categories related to clinical outcome.

PATIENTS AND METHODS Samples of 44 patients with JMML were available for microarray gene expression analysis. A diagnostic classification (DC) model developed for leukemia and myelodysplastic syndrome classification was used to classify the specimens and identify prognostically relevant categories. Statistical analysis was performed to determine the prognostic value of the classification and the genes identifying prognostic categories were further analyzed through R software.

RESULTS The samples could be divided into two major groups: 20 specimens were classified as acute myeloid leukemia (AML) -like and 20 samples as nonAML-like. Four patients could not be assigned to a unique class. The 10-year probability of survival after diagnosis of AML-like and nonAML-like patients was significantly different (7% v 74%; P = .0005). Similarly, the 10-year event-free survival after HSCT was 6% for AML-like and 63% for nonAML-like patients (P = .0010).

CONCLUSION Gene expression-based classification identifies two groups of patients with JMML with distinct prognosis outperforming all known clinical parameters in terms of prognostic relevance. Gene expression-based classification could thus be prospectively used to guide clinical/therapeutic decisions.

6. Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in Advanced-stage diffuse large B-Cell lymphoma.

J Clin Oncol. 2010 Apr 10;28(11):1896-903. Epub 2010 Mar 8.

Moskowitz CH, Schöder H, Teruya-Feldstein J, Sima C, Iasonos A, Portlock CS, Straus D, Noy A, Palomba ML, O'Connor OA, Horwitz S, Weaver SA, Meikle JL, Filippa DA, Caravelli JF, Hamlin PA, Zelenetz AD.

PURPOSE: In studies of diffuse large B-cell lymphoma, positron emission tomography with [(18)F]fluorodeoxyglucose (FDG-PET) performed after two to four cycles of chemotherapy has demonstrated

prognostic significance. However, some patients treated with immunochemotherapy experience a favorable long-term outcome despite a positive interim FDG-PET scan. To clarify the significance of interim FDG-PET scans, we prospectively studied interim FDG-positive disease within a risk-adapted sequential immunochemotherapy program.

PATIENTS AND METHODS: From March 2002 to November 2006, 98 patients at Memorial Sloan-Kettering Cancer Center received induction therapy with four cycles of accelerated R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisone) followed by an interim FDG-PET scan. If the FDG-PET scan was negative, patients received three cycles of ICE (ifosfamide, carboplatin, and etoposide) consolidation therapy. If residual FDG-positive disease was seen, patients underwent biopsy; if the biopsy was negative, they also received three cycles of ICE. Patients with a positive biopsy received ICE followed by autologous stem-cell transplantation.

RESULTS: At a median follow-up of 44 months, overall and progression-free survival were 90% and 79%, respectively. Ninety-seven patients underwent interim FDG-PET scans; 59 had a negative scan, 51 of whom are progression free. Thirty-eight patients with FDG-PET-positive disease underwent repeat biopsy; 33 were negative, and 26 remain progression free after ICE consolidation therapy. Progression-free survival of interim FDG-PET-positive/biopsy-negative patients was identical to that in patients with a negative interim FDG-PET scan (P = .27).

CONCLUSION: Interim or post-treatment FDG-PET evaluation did not predict outcome with this dose-dense, sequential immunochemotherapy program. Outside of a clinical trial, we recommend biopsy confirmation of an abnormal interim FDG-PET scan before changing therapy.

7. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells.

Blood. 2010 Apr 8;115(14):2960-70. Epub 2010 Jan 26.

Moosmann A, Bigalke I, Tischer J, Schirrmann L, Kasten J, Tippmer S, Leeping M, Prevalsek D, Jaeger G, Ledderose G, Mautner J, Hammerschmidt W, Schendel DJ, Kolb HJ.

Posttransplantation lymphoproliferative disease (PTLD) associated with Epstein-Barr virus (EBV) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation. PTLD is efficiently prevented by adoptive transfer of EBV-specific T cells from the donor. To make EBV-specific T cells available in urgent clinical situations, we developed a rapid protocol for their isolation by overnight stimulation of donor blood cells with peptides derived from 11 EBV antigens, interferon-gamma surface capture, and immunomagnetic separation. Six patients with PTLD received 1 transfusion of EBV-specific T cells. No response was seen in 3 patients who had late-stage disease with multiorgan dysfunction at the time of T-cell transfer. In 3 patients who received T cells at an earlier stage of disease, we observed complete and stable remission of PTLD. Two patients have remained free from EBV-associated disease for more than 2 years. CD8(+) T cells specific for EBV early antigens rapidly expanded after T-cell transfer, temporarily constituted greater than 20% of all peripheral blood lymphocytes, and were maintained throughout the observation period. Thus, a rapid and sustained reconstitution of a protective EBV-specific T-cell memory occurred after the infusion of small numbers of directly isolated EBV-specific T cells.

8. Bortezomib, thalidomide, dexamethasone induction therapy followed by melphalan, prednisolone, thalidomide consolidation therapy as a first line of treatment for patients with multiple myeloma who are non-transplant candidates: results of the Korean Multiple Myeloma Working Party (KMMWP).

Ann Hematol. 2010 May;89(5):489-97. Epub 2009 Dec 10.

Eom HS, Kim YK, Chung JS, Kim K, Kim HJ, Kim HY, Jin JY, Do YR, Oh SJ, Suh C, Seong CM, Kim CS, Lee DS, Lee JH.

Bortezomib (VELCADE), thalidomide and dexamethasone (VTD), as well as melphalan, prednisolone, and thalidomide (MPT) therapy, are highly effective in patients with multiple myeloma. We evaluated the responses and survival times of 35 patients treated with VTD followed by MPT. All patients were newly diagnosed and non-transplantation candidates. Patients received six cycles of VTD, which were followed by eight cycles of MPT.

Approximately 97% of patients exhibited early responses to therapy, as early as the second cycle of VTD. Thirty percent of the responses were high quality, which was defined as a complete response (CR), a near-CR or a very good partial response. High-risk patients were defined as patients with any of the following aberrations: del(13), t(4;14), or del(17p). The remaining patients were defined as standard risk. Eleven high-risk patients showed 100% response rates, including 91% high-quality responses. In contrast, 13 standard-risk patients exhibited 92% response rates, including 61% high-quality responses. The overall 2-year survival rates were 60% in high-risk patients and 85% in standard-risk patients, which was not significantly different. As a first-line therapy, VTD followed by MPT has the potential to provide high-quality responses with durable remission among elderly and high-risk patients (clinicaltrials.gov identifier: NCT00320476).

9. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial.

Exp Neurol. 2010 May;223(1):229-37. Epub 2009 Aug 13.

Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K, Testa L, Stecco A, Tarletti R, Miglioretti M, Fava E, Nasuelli N, Cisari C, Massara M, Vercelli R, Oggioni GD, Carriero A, Cantello R, Monaco F, Fagioli F.

Amyotrophic Lateral Sclerosis (ALS) is a devastating incurable disease. Stem-cell-based therapies represent a new possible strategy for ALS clinical research. The objectives of this Phase 1 clinical study were to assess the feasibility and toxicity of mesenchymal stem cell transplantation and to test the impact of a cell therapy in ALS patients. The trial was approved and monitored by the National Institute of Health and by the Ethics Committees of all participating Institutions. Autologous MSCs were isolated from bone marrow, expanded in vitro and analyzed according to GMP conditions. Expanded MSCs were suspended in the autologous cerebrospinal fluid (CSF) and directly transplanted into the spinal cord at a high thoracic level with a surgical procedure. Ten ALS patients were enrolled and regularly monitored before and after transplantation by clinical, psychological, neuroradiological and neurophysiological assessments. There was no immediate or delayed transplant-related toxicity. Clinical, laboratory, and radiographic evaluations of the patients showed no serious transplant-related adverse events. Magnetic resonance images (MRI) showed no structural changes (including tumor formation) in either the brain or the spinal cord. However the lack of post mortem material prevents any definitive conclusion about the vitality of the MSCs after transplantation. In conclusion, this study confirms that MSC transplantation into the spinal cord of ALS patients is safe and that MSCs might have a clinical use for future ALS cell based clinical trials.

STEM CELL TRANSPLANTATION

1. Hematopoietic stem cell transplantation: a global perspective.

JAMA. 2010 Apr 28;303(16):1617-24.

Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, Szer J, Lipton J, Schwendener A, Gratwohl M, Frauendorfer K, Niederwieser D, Horowitz M, Kodera Y; Worldwide Network of Blood and Marrow Transplantation.

CONTEXT: Hematopoietic stem cell transplantation (HSCT) requires significant infrastructure. Little is known about HSCT use and the factors associated with it on a global level.

OBJECTIVES: To determine current use of HSCT to assess differences in its application and to explore associations of macroeconomic factors with transplant rates on a global level.

DESIGN, SETTING, AND PATIENTS: Retrospective survey study of patients receiving allogeneic and autologous HSCTs for 2006 collected by 1327 centers in 71 participating countries of the Worldwide Network for Blood and Marrow Transplantation. The regional areas used herein are (1) the Americas (the corresponding World Health Organization regions are North and South America); (2) Asia (Southeast Asia and the Western Pacific Region, which includes Australia and New Zealand); (3) Europe (includes Turkey and Israel); and (4) the Eastern Mediterranean and Africa.

MAIN OUTCOME MEASURES: Transplant rates (number of HSCTs per 10 million inhabitants) by indication,

donor type, and country; description of main differences in HSCT use; and macroeconomic factors of reporting countries associated with HSCT rates. RESULTS: There were 50 417 first HSCTs; 21 516 allogeneic (43%) and 28 901 autologous (57%). The median HSCT rates varied between regions and countries from 48.5 (range, 2.5-505.4) in the Americas, 184 (range, 0.6-488.5) in Asia, 268.9 (range, 5.7-792.1) in Europe, and 47.7 (range, 2.8-95.3) in the Eastern Mediterranean and Africa. No HSCTs were performed in countries with less than 300,000 inhabitants, smaller than 960 km(2), or having less than US $680 gross national income per capita. Use of allogeneic or autologous HSCT, unrelated or family donors for allogeneic HSCT, and proportions of disease indications varied significantly between countries and regions. In linear regression analyses, government health care expenditures (r(2) = 77.33), HSCT team density (indicates the number of transplant teams per 1 million inhabitants; r(2) = 76.28), human development index (r(2) = 74.36), and gross national income per capita (r(2) = 74.04) showed the highest associations with HSCT rates.

CONCLUSION: Hematopoietic stem cell transplantation is used for a broad spectrum of indications worldwide, but most frequently in countries with higher gross national incomes, higher governmental health care expenditures, and higher team densities.

2. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma.

Cancer Cell. 2010 Apr 13;17(4):362-75.

Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, Modrusan Z, Nacu S, Guerrero S, Edgar KA, Wallin JJ, Lamszus K, Westphal M, Heim S, James CD, VandenBerg SR, Costello JF, Moorefield S, Cowdrey CJ, Prados M, Phillips HS.

The neural stem cell marker CD133 is reported to identify cells within glioblastoma (GBM) that can initiate neurosphere growth and tumor formation; however, instances of CD133(-) cells exhibiting similar properties have also been reported. Here, we show that some PTEN-deficient GBM tumors produce a series of CD133(+) and CD133(-) self-renewing tumor-initiating cell types and provide evidence that these cell types constitute a lineage hierarchy. Our results show that the capacities for self-renewal and tumor initiation in GBM need not be restricted to a uniform population of stemlike cells, but can be shared by a lineage of self-renewing cell types expressing a range of markers of forebrain lineage.

3. Implantation of ferumoxides labeled human mesenchymal stem cells in cartilage defects.

J Vis Exp. 2010 Apr 5;(38). pii: 1793. doi: 10.3791/1793.

Nedopil AJ, Mandrussow LG, Daldrup-Link HE.

The field of tissue engineering integrates the principles of engineering, cell biology and medicine towards the regeneration of specific cells and functional tissue. Matrix associated stem cell implants (MASI) aim to regenerate cartilage defects due to arthritic or traumatic joint injuries. Adult mesenchymal stem cells (MSCs) have the ability to differentiate into cells of the chondrogenic lineage and have shown promising results for cell-based articular cartilage repair technologies. Autologous MSCs can be isolated from a variety of tissues, can be expanded in cell cultures without losing their differentiation potential, and have demonstrated chondrogenic differentiation in vitro and in vivo(1, 2). In order to provide local retention and viability of transplanted MSCs in cartilage defects, a scaffold is needed, which also supports subsequent differentiation and proliferation. The architecture of the scaffold guides tissue formation and permits the extracellular matrix, produced by the stem cells, to expand. Previous investigations have shown that a 2% agarose scaffold may support the development of stable hyaline cartilage and does not induce immune responses(3). Long term retention of transplanted stem cells in MASI is critical for cartilage regeneration. Labeling of MSCs with iron oxide nanoparticles allows for long-term in vivo tracking with non-invasive MR imaging techniques(4). This presentation will demonstrate techniques for labeling MSCs with iron oxide nanoparticles, the generation of cell-agarose constructs and implantation of these constructs into cartilage defects. The labeled constructs can be tracked non-invasively with MR-Imaging.

4. Cytogenetic studies in acute leukemia patients relapsing after allogeneic stem cell transplantation.

Cancer Genet Cytogenet. 2010 Apr 15;198(2):135-43.

Schmidt-Hieber M, Blau IW, Richter G, Türkmen S, Bommer C, Thiel G, Neitzel H, Stroux A, Uharek L, Thiel E, Blau O.

We analyzed karyotype stability in 22 patients with acute leukemia at relapse or disease progression after allogeneic stem cell transplantation (allo-SCT). Karyotypes before and at relapse after allo-SCT were different in 15 patients (68%), the most frequent type being clonal evolution either alone or combined with clonal devolution (13 patients). Patients with and without a karyotype change did not differ significantly in overall survival (OS) (median, 399 vs. 452 days; P = 0.889) and survival after relapse (median, 120 vs. 370 days; P = 0.923). However, acquisition of additional structural chromosome 1 abnormalities at relapse after allo-SCT occurred more frequently than expected and was associated with reduced OS (median, 125 vs. 478 days; P = 0.008) and shorter survival after relapse (median, 37 vs. 370 days; P = 0.002). We identified a previously undescribed clonal evolution involving t(15;17) without PML-RARA rearrangement in an AML patient. We conclude that a karyotype change is common at relapse after allo-SCT in acute leukemia patients. Moreover, our data suggest that additional structural chromosome 1 abnormalities are overrepresented at relapse after allo-SCT in these patients and, in contrast to a karyotype change per se, are associated with reduced OS and shorter survival after relapse.

5. Ovarian cancer stem-like side-population cells are tumourigenic and chemoresistant.

Br J Cancer. 2010 Apr 13;102(8):1276-83. Epub 2010 Mar 30.

Hu L, McArthur C, Jaffe RB.

BACKGROUND: Ovarian cancer is the most lethal gynaecological malignancy. Although ovarian cancer patients often respond initially to chemotherapy, they usually develop chemoresistance. We hypothesised that a small portion of ovarian cancer cells have stem-like cell properties that contribute to tumourigenesis and drug resistance.

METHODS: Flow cytometry and Hoechst 33342 efflux isolated side-population (SP) cells from ascites derived from ovarian cancer patients and from mice inoculated with human ovarian cancer cell lines. The SP cells were examined for stem cell markers OCT4, NANOG, STELLAR, and ABCG2/BCRP1 by immunocytochemistry and RT-PCR. The SP cells and non-SP cells were studied for tumourigenesis and chemoresistance in vitro and in vivo.

RESULTS: The SP cells expressed ABCG2/BCRP1, OCT4, STELLAR, and NANOG, detected by immunocytochemistry and RT-PCR. ABCG2/BCRP1 expression was higher in SP than in non-SP cells. Xenogeneic mice inoculated with SP cells yielded more tumours than did mice inoculated with non-SP cells. In parallel, SP cell culture resulted in extensive cell proliferation, which was markedly more than in non-SP cells. SP cells resisted chemotherapy compared with non-SP cells, both in vivo and in vitro.

CONCLUSION: Ovarian cancer SP cells are tumourigenic and chemoresistant. ABCG2/BCRP1 has an important role in chemoresistance, which has implications for new therapeutic approaches.

6. Respiratory virus pneumonia after hematopoietic cell transplantation (HCT): associations between viral load in bronchoalveolar lavage samples, viral RNA detection in serum samples, and clinical outcomes of HCT.

J Infect Dis. 2010 May 1;201(9):1404-13.

Campbell AP, Chien JW, Kuypers J, Englund JA, Wald A, Guthrie KA, Corey L, Boeckh M.

BACKGROUND: Few data exist on respiratory virus quantitation in lower respiratory samples and detection in serum from hematopoietic cell transplant (HCT) recipients with respiratory virus-associated pneumonia.

METHODS: We retrospectively identified HCT recipients with respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, metapneumovirus (MPV), and coronavirus (CoV) detected in bronchoalveolar lavage (BAL) samples, and we tested stored BAL and/or serum samples by quantitative polymerase chain reaction.

RESULTS: In 85 BAL samples from 82 patients, median viral loads were as follows: for RSV (n = 35), 2.6 x 10(6) copies/mL; for parainfluenza virus (n = 35), 4.9 x 10(7) copies/mL; for influenza virus (n = 9), 6.8 x 10(5) copies/mL; for MPV (n = 7), 3.9 x 10(7) copies/mL; and for CoV (n = 4), 1.8 x 10(5) copies/mL. Quantitative viral load was not associated with mechanical ventilation or death. Viral RNA was detected in serum samples from 6 of 66 patients: 4 of 41 with RSV pneumonia, 1 with influenza B, and 1 with MPV/influenza A virus/CoV coinfection (influenza A virus and MPV RNA detected). RSV detection in serum was associated with high viral load in BAL samples (p = .05), and viral RNA detection in serum was significantly associated with death (adjusted rate ratio, 1.8; (p = .02).

CONCLUSION: Quantitative polymerase chain reaction detects high viral loads in BAL samples from HCT recipients with respiratory virus pneumonia. Viral RNA is also detectable in the serum of patients with RSV, influenza, and MPV pneumonia and may correlate with the severity of disease.

7. Expansion of CD133(+) colon cancer cultures retaining stem cell properties to enable cancer stem cell target discovery.

Br J Cancer. 2010 Apr 13;102(8):1265-75. Epub 2010 Mar 23.

Fang DD, Kim YJ, Lee CN, Aggarwal S, McKinnon K, Mesmer D, Norton J, Birse CE, He T, Ruben SM, Moore PA.

BACKGROUND: Despite earlier studies demonstrating in vitro propagation of solid tumour cancer stem cells (CSCs) as non-adherent tumour spheres, it remains controversial as to whether CSCs can be maintained in vitro. Additional validation of the CSC properties of tumour spheres would support their use as CSC models and provide an opportunity to discover additional CSC cell surface markers to aid in CSC detection and potential elimination.

METHODS: Primary tumour cells isolated from 13 surgically resected colon tumour specimens were propagated using serum-free CSC-selective conditions. The CSC properties of long-term cultured tumour spheres were established and mass spectrometry-based proteomics performed.

RESULTS: Freshly isolated CD133(+) colorectal cancer cells gave rise to long-term tumour sphere (or spheroids) cultures maintaining CD133 expression. These spheroid cells were able to self-renew and differentiate into adherent epithelial lineages and recapitulate the phenotype of the original tumour. Relative to their differentiated progeny, tumour spheroid cells were more resistant to the chemotherapeutic irinotecan. Finally, CD44, CD166, CD29, CEACAM5, cadherin 17, and biglycan were identified by mass spectrometry to be enriched in CD133(+) tumour spheroid cells.

CONCLUSION: Our data suggest that ex vivo-expanded colon CSCs isolated from clinical specimens can be maintained in culture enabling the identification of CSC cell surface-associated proteins.

8. Impact of EWS-ETS fusion type on disease progression in Ewing's sarcoma/peripheral primitive neuroectodermal tumor: prospective results from the cooperative Euro-E.W.I.N.G. 99 trial.

J Clin Oncol. 2010 Apr 20;28(12):1982-8. Epub 2010 Mar 22. Comment in: J Clin Oncol. 2010 Apr 20;28(12):1973-4.

Le Deley MC, Delattre O, Schaefer KL, Burchill SA, Koehler G, Hogendoorn PC, Lion T, Poremba C, Marandet J, Ballet S, Pierron G, Brownhill SC, Nesslböck M, Ranft A, Dirksen U, Oberlin O, Lewis IJ, Craft AW, Jürgens H, Kovar H.

PURPOSE: EWS-ETS fusion genes are the driving force in Ewing's sarcoma pathogenesis. Because of the variable breakpoint locations in the involved genes, there is heterogeneity in fusion RNA and protein architecture. Since previous retrospective studies suggested prognostic differences among patients expressing

different EWS-FLI1 fusion types, the impact of fusion RNA architecture on disease progression and relapse was studied prospectively within the Euro-E.W.I.N.G. 99 clinical trial.

PATIENTS AND METHODS: Among 1,957 patients who registered before January 1, 2007, 703 primary tumors were accessible for the molecular biology study. Fusion type was assessed by polymerase chain reaction on frozen (n = 578) or paraffin-embedded materials (n = 125). The primary end point was the time to disease progression or relapse. Results After exclusion of noninformative patients, 565 patients were entered into the prognostic factor analysis comparing type 1 (n = 296), type 2 (n = 133), nontype 1/nontype 2 EWS-FLI1 (n = 91) and EWS-ERG fusions (n = 45). Median follow-up time was 4.5 years. The distribution of sex, age, tumor volume, tumor site, disease extension, or histologic response did not differ between the four fusion type groups. We did not observe any significant prognostic value of the fusion type on the risk of progression or relapse. The only slight difference was that the risk of progression or relapse associated with nontype 1/nontype 2 EWS-FLI1 fusions was 1.38 (95% CI, 0.96 to 2.0) times higher than risk associated with other fusion types, but it was not significant (P = .10).

CONCLUSION: In contrast to retrospective studies, the prospective evaluation did not confirm a prognostic benefit for type 1 EWS-FLI1 fusions.

9. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma.

J Clin Oncol. 2010 Apr 20;28(12):2077-84. Epub 2010 Mar 22.

Ladetto M, Pagliano G, Ferrero S, Cavallo F, Drandi D, Santo L, Crippa C, De Rosa L, Pregno P, Grasso M, Liberati AM, Caravita T, Pisani F, Guglielmelli T, Callea V, Musto P, Cangialosi C, Passera R, Boccadoro M, Palumbo A.

PURPOSE We investigated the effect on minimal residual disease, by qualitative and real-time quantitative polymerase chain reaction (RQ-PCR), of a consolidation regimen that included bortezomib, thalidomide, and dexamethasone (VTD) in patients with multiple myeloma (MM) responding to autologous stem-cell transplantation (auto-SCT).

PATIENTS AND METHODS Patients achieving at least very good partial response who had an available molecular marker based on the immunoglobulin heavy-chain rearrangement received four courses of treatment every month: four infusions per month of bortezomib at 1.6 mg/m(2), thalidomide at 200 mg/d, and dexamethasone at 20 mg/d on days 1 to 4, 8 to 11, and 15 to 18. Patients were studied with tumor-clone-specific primers by qualitative nested PCR and RQ-PCR. Results Of 39 patients enrolled, 31 received the four VTD courses. Immunofixation complete responses increased from 15% after auto-SCT to 49% after VTD. Molecular remissions (MRs) were 3% after auto-SCT and 18% after VTD. Median time to maximum response was 3.5 months. So far, no patient in MR has relapsed (median follow-up, 42 months). VTD consolidation induced an additional depletion of 4.14 natural logarithms of tumor burden by RQ-PCR. Patients with a tumor load less than the median value after VTD had outcomes better than those who had tumor loads above the median value after VTD (at median follow-up: progression-free survival, 100% v 57%; P < .001).

CONCLUSION To the best of our knowledge, this study is the first to document the occurrence of persistent MRs in a proportion of MM patients treated without allogeneic transplantation. Moreover, the major reduction in tumor load recorded by RQ-PCR after VTD suggests that unprecedented levels of tumor cell reduction can be achieved in MM thanks to the new nonchemotherapeutic drugs.

10. Lymphoma recurrence 5 years or later following diffuse large B-cell lymphoma: clinical characteristics and outcome.

J Clin Oncol. 2010 Apr 20;28(12):2094-100. Epub 2010 Mar 22.

Larouche JF, Berger F, Chassagne-Clément C, Ffrench M, Callet-Bauchu E, Sebban C, Ghesquières H, Broussais-Guillaumot F, Salles G, Coiffier B.

PURPOSE Patients with diffuse large B-cell lymphoma (DLBCL) usually relapse early following diagnosis but some relapses happen at 5 years or later. Few data exist regarding clinical characteristics and outcome of these

patients.

PATIENTS AND METHODS We performed a retrospective analysis of all patients from two centers in Lyon, France, between 1985 and 2003 who had a biopsy-proven relapse 5 years or later following diagnosis of DLBCL. All available biopsies were reviewed and immunohistochemistry was completed.

RESULTS Among 1,492 patients with DLBCL, 54 were eligible. At diagnosis, 63% of patients had stage I-II, 82% had low/low-intermediate International Prognostic Index (IPI) score, 65% had extranodal involvement, 24% had an indolent component associated with DLBCL, 57% had germinal center phenotype, and 43% had non-germinal center phenotype. Median time from diagnosis to relapse was 7.4 years (range, 5 to 20.5 years). At time of relapse, 83% had DLBCL histology, and 17% had indolent histology. Having an indolent component at diagnosis was associated with indolent histology at relapse (P = .028). Five-year event free-survival (EFS) was 17% for patients with DLBCL relapse and 61% for patients with indolent relapse (P = .027). Five-year overall survival was 27% for patients with DLBCL and 75% for patients with indolent relapse (P = .029). For DLBCL relapse, 3-year EFS was 56% versus 18% with autologous stem-cell transplantation or not, respectively (P = .0661).

CONCLUSION Patients with DLBCL who had a late relapse usually had localized stage, favorable IPI score, and extranodal involvement at diagnosis. The outcome of patients with DLBCL at time of relapse remains poor, and aggressive treatment such as autologous stem-cell transplantation should be pursued whenever possible. Biopsy at relapse is essential because some patients relapse with indolent histology.

11. High EVI1 expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities.

J Clin Oncol. 2010 Apr 20;28(12):2101-7. Epub 2010 Mar 22.

Gröschel S, Lugthart S, Schlenk RF, Valk PJ, Eiwen K, Goudswaard C, van Putten WJ, Kayser S, Verdonck LF, Lübbert M, Ossenkoppele GJ, Germing U, Schmidt-Wolf I, Schlegelberger B, Krauter J, Ganser A, Döhner H, Löwenberg B, Döhner K, Delwel R.

PURPOSE The purpose of this study was to investigate frequency and prognostic significance of high EVI1 expression in acute myeloid leukemia (AML).

PATIENTS AND METHODS A diagnostic assay detecting multiple EVI1 splice variants was developed to determine the relative EVI1 expression by single real-time quantitative polymerase chain reaction in 1,382 newly diagnosed adult patients with AML younger than 60 years. Patients were treated on four Dutch-Belgian HOVON (n = 458) and two German-Austrian AML Study Group protocols (n = 924).

RESULTS The EVI1 assay was tested in the HOVON cohort and validated in the AMLSG cohort. High EVI1 levels (EVI1(+)) were found with similar frequencies in both cohorts combined, with a 10.7% incidence (148 of 1,382). EVI1(+) independently predicted low complete remission (CR) rate (odds ratio, 0.54; P = .002), adverse relapse-free survival (RFS; hazard ratio [HR], 1.32; P = .05), and event-free survival (EFS; HR, 1.46; P < .001). This adverse prognostic impact was more pronounced in the intermediate cytogenetic risk group (EFS; HR, 1.64; P < .001; and RFS; HR, 1.55; P = .02), and was also apparent in cytogenetically normal AML (EFS; HR, 1.67; P = .008). Besides inv(3)/t(3;3), EVI1(+) was significantly associated with chromosome abnormalities monosomy 7 and t(11q23), conferring prognostic impact within these two cytogenetic subsets. EVI1(+) was virtually absent in favorable-risk AML and AML with NPM1 mutations. Patients with EVI1(+) AML (n = 28) who received allogeneic stem cell transplantation in first CR had significantly better 5-year RFS (33% +/- 10% v 0%).

CONCLUSION EVI1 expression in AML is unequally distributed in cytogenetic subtypes. It predicts poor outcome, particularly among intermediate cytogenetic risk AML. Patients with EVI1(+) AML may benefit from allogeneic transplantation in first CR. Pretreatment EVI1 screening should be included in risk stratification.

12. Gene expression-based classification as an independent predictor of clinical outcome in juvenile myelomonocytic leukemia.

J Clin Oncol. 2010 Apr 10;28(11):1919-27. Epub 2010 Mar 15.

Bresolin S, Zecca M, Flotho C, Trentin L, Zangrando A, Sainati L, Stary J, de Moerloose B, Hasle H, Niemeyer CM, Te Kronnie G, Locatelli F, Basso G.

PURPOSE Juvenile myelomonocytic leukemia (JMML) is a rare early childhood myelodysplastic/myeloproliferative disorder characterized by an aggressive clinical course. Age and hemoglobin F percentage at diagnosis have been reported to predict both survival and outcome after hematopoietic stem cell transplantation (HSCT). However, no genetic markers with prognostic relevance have been identified so far. We applied gene expression-based classification to JMML samples in order to identify prognostic categories related to clinical outcome.

PATIENTS AND METHODS Samples of 44 patients with JMML were available for microarray gene expression analysis. A diagnostic classification (DC) model developed for leukemia and myelodysplastic syndrome classification was used to classify the specimens and identify prognostically relevant categories. Statistical analysis was performed to determine the prognostic value of the classification and the genes identifying prognostic categories were further analyzed through R software.

RESULTS The samples could be divided into two major groups: 20 specimens were classified as acute myeloid leukemia (AML) -like and 20 samples as nonAML-like. Four patients could not be assigned to a unique class. The 10-year probability of survival after diagnosis of AML-like and nonAML-like patients was significantly different (7% v 74%; P = .0005). Similarly, the 10-year event-free survival after HSCT was 6% for AML-like and 63% for nonAML-like patients (P = .0010).

CONCLUSION Gene expression-based classification identifies two groups of patients with JMML with distinct prognosis outperforming all known clinical parameters in terms of prognostic relevance. Gene expression-based classification could thus be prospectively used to guide clinical/therapeutic decisions.

13. Effect of age on outcome of reduced-intensity hematopoietic cell transplantation for older patients with acute myeloid leukemia in first complete remission or with myelodysplastic syndrome.

J Clin Oncol. 2010 Apr 10;28(11):1878-87. Epub 2010 Mar 8.

McClune BL, Weisdorf DJ, Pedersen TL, Tunes da Silva G, Tallman MS, Sierra J, Dipersio J, Keating A, Gale RP, George B, Gupta V, Hahn T, Isola L, Jagasia M, Lazarus H, Marks D, Maziarz R, Waller EK, Bredeson C, Giralt S.

PURPOSE Acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) primarily afflict older individuals. Hematopoietic cell transplantation (HCT) is generally not offered because of concerns of excess morbidity and mortality. Reduced-intensity conditioning (RIC) regimens allow increased use of allogeneic HCT for older patients. To define prognostic factors impacting long-term outcomes of RIC regimens in patients older than age 40 years with AML in first complete remission or MDS and to determine the impact of age, we analyzed data from the Center for International Blood and Marrow Transplant Research (CIBMTR).

PATIENTS AND METHODS We reviewed data reported to the CIBMTR (1995 to 2005) on 1,080 patients undergoing RIC HCT. Outcomes analyzed included neutrophil recovery, incidence of acute or chronic graft-versus-host disease (GVHD), nonrelapse mortality (NRM), relapse, disease-free survival (DFS), and overall survival (OS).

RESULTS Univariate analyses demonstrated no age group differences in NRM, grade 2 to 4 acute GVHD, chronic GVHD, or relapse. Patients age 40 to 54, 55 to 59, 60 to 64, and > or = 65 years had 2-year survival rates as follows: 44% (95% CI, 37% to 52%), 50% (95% CI, 41% to 59%), 34% (95% CI, 25% to 43%), and 36% (95% CI, 24% to 49%), respectively, for patients with AML (P = .06); and 42% (95% CI, 35% to 49%), 35% (95% CI, 27% to 43%), 45% (95% CI, 36% to 54%), and 38% (95% CI, 25% to 51%), respectively, for patients with MDS (P = .37). Multivariate analysis revealed no significant impact of age on NRM, relapse, DFS, or OS (all P > .3). Greater HLA disparity adversely affected 2-year NRM, DFS, and OS. Unfavorable cytogenetics adversely impacted relapse, DFS, and OS. Better pre-HCT performance status predicted improved 2-year OS.

CONCLUSION With these similar outcomes observed in older patients, we conclude that older age alone should not be considered a contraindication to HCT.

14. Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in Advanced-stage diffuse large B-Cell lymphoma.

J Clin Oncol. 2010 Apr 10;28(11):1896-903. Epub 2010 Mar 8.

Moskowitz CH, Schöder H, Teruya-Feldstein J, Sima C, Iasonos A, Portlock CS, Straus D, Noy A, Palomba ML, O'Connor OA, Horwitz S, Weaver SA, Meikle JL, Filippa DA, Caravelli JF, Hamlin PA, Zelenetz AD.

PURPOSE In studies of diffuse large B-cell lymphoma, positron emission tomography with [(18)F]fluorodeoxyglucose (FDG-PET) performed after two to four cycles of chemotherapy has demonstrated prognostic significance. However, some patients treated with immunochemotherapy experience a favorable long-term outcome despite a positive interim FDG-PET scan. To clarify the significance of interim FDG-PET scans, we prospectively studied interim FDG-positive disease within a risk-adapted sequential immunochemotherapy program.

PATIENTS AND METHODS From March 2002 to November 2006, 98 patients at Memorial Sloan-Kettering Cancer Center received induction therapy with four cycles of accelerated R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisone) followed by an interim FDG-PET scan. If the FDG-PET scan was negative, patients received three cycles of ICE (ifosfamide, carboplatin, and etoposide) consolidation therapy. If residual FDG-positive disease was seen, patients underwent biopsy; if the biopsy was negative, they also received three cycles of ICE. Patients with a positive biopsy received ICE followed by autologous stem-cell transplantation.

RESULTS At a median follow-up of 44 months, overall and progression-free survival were 90% and 79%, respectively. Ninety-seven patients underwent interim FDG-PET scans; 59 had a negative scan, 51 of whom are progression free. Thirty-eight patients with FDG-PET-positive disease underwent repeat biopsy; 33 were negative, and 26 remain progression free after ICE consolidation therapy. Progression-free survival of interim FDG-PET-positive/biopsy-negative patients was identical to that in patients with a negative interim FDG-PET scan (P = .27).

CONCLUSION Interim or post-treatment FDG-PET evaluation did not predict outcome with this dose-dense, sequential immunochemotherapy program. Outside of a clinical trial, we recommend biopsy confirmation of an abnormal interim FDG-PET scan before changing therapy.

15. Relapse and late mortality in 5-year survivors of myeloablative allogeneic hematopoietic cell transplantation for chronic myeloid leukemia in first chronic phase.

J Clin Oncol. 2010 Apr 10;28(11):1888-95. Epub 2010 Mar 8.

Goldman JM, Majhail NS, Klein JP, Wang Z, Sobocinski KA, Arora M, Horowitz MM, Rizzo JD.

PURPOSE Allogeneic hematopoietic cell transplantation (HCT) is curative therapy for chronic myeloid leukemia (CML), but its long-term outcomes are not well described. We studied the long-term outcomes of CML patients in first chronic phase who receive an allogeneic HCT.

PATIENTS AND METHODS Our study included 2,444 patients who received myeloablative HCT for CML in first chronic phase between 1978 and 1998 and survived in continuous complete remission for at least 5 years (median follow-up, 11 years; range, 5 to 25 years). Donor sources were human leukocyte antigen-matched siblings in 1,692 patients, unrelated donors in 639 patients, and other related donors in 113 patients.

RESULTS Overall survival rates at 15 years were 88% (95% CI, 86% to 90%) for sibling HCT and 87% (95% CI, 83% to 90%) for unrelated donor HCT. Corresponding cumulative incidences of relapse were 8% (95% CI, 7% to 10%) and 2% (95% CI, 1% to 4%), respectively. The latest relapse was reported 18 years post-HCT. In multivariable analyses, history of chronic graft-versus-host disease increased risks of late overall mortality and nonrelapse mortality but reduced risks of relapse. In comparison with age-, race-, and sex-adjusted normal populations, the mortality of HCT recipients was significantly higher until 14 years post-HCT; thereafter, mortality rates were similar to those of the general population (relative mortality ratio at 15 years, 2.3; 95% CI, 0 to 4.9).

CONCLUSION Recipients of allogeneic HCT for CML in first chronic phase who remain in remission for at least

5 years have favorable subsequent long-term survival, and their mortality rates eventually approach those of the general population.

16. Syndrome of inappropriate secretion of antidiuretic hormone as a leading cause of hyponatremia in children who underwent chemotherapy or stem cell transplantation.

Pediatr Blood Cancer. 2010 May;54(5):734-7.

Lim YJ, Park EK, Koh HC, Lee YH.

BACKGROUND: Hyponatremia is a common metabolic disorder in cancer patients. However, little information is available for patients receiving chemotherapy or stem cell transplantation (SCT). We analyzed the frequency, characteristics, and various causes of hyponatremia including routine use of hypotonic fluids in children following chemotherapy or SCT.

PROCEDURE: We reviewed the clinical and laboratory data of 63 children who received chemotherapy or SCT at the Department of Pediatrics, Hanyang University Medical Center from July 2005 to July 2008.

RESULTS: All 63 patients at admission received routine parenteral fluids of 0.25% or 0.45% NaCl and 82 episodes of hyponatremia were observed in 40 (63.5%) patients. Of these 82 episodes, 50 episodes of hyponatremia developed in 29 children following chemotherapy and 32 episodes in 16 children following SCT. Seventy-six out of 82 episodes (92.7%) of hyponatremia developed in 37 patients receiving hypotonic fluids with NaCl concentrations between 30 and 150 mEq/L. The frequency of SIADH in the SCT setting was more frequent (14/21, 66.6%) than in the chemotherapy setting (18/58, 31.0%) (P = 0.02), even though the leading cause of hyponatremia was SIADH in both settings.

CONCLUSIONS: SIADH is a leading cause of hyponatremia in children following chemotherapy or SCT, and more frequent in SCT settings than in chemotherapy settings. Furthermore, the routine use of hypotonic fluids which could aggravate the development of hyponatremia for these patients should be avoided and then switched to isotonic fluids.

17. Remission re-induction chemotherapy with clofarabine, topotecan, thiotepa, and vinorelbine for patients with relapsed or refractory leukemia.

Pediatr Blood Cancer. 2010 May;54(5):687-93.

Steinherz PG, Shukla N, Kobos R, Steinherz L.

BACKGROUND: We determined the maximum tolerated dose (MTD) of clofarabine when administered with topotecan, vinorelbine, thiotepa, and dexamethasone (TVTC) for children with relapsed or refractory acute leukemia, and observed the efficacy and toxicities of this therapy. PROCEDURE: Twelve patients with acute lymphoblastic or myeloblastic leukemia were given a 14-day remission induction therapy. Clofarabine was administered at a dose of 30 or 40 mg/m(2)/day over 2 hr for five consecutive days in six patients each. Patients who achieved a remission proceeded to a stem cell transplant (HSCT). A second cycle could be administered prior to HSCT. RESULTS: Of the six patients at the 30 mg/m(2) clofarabine dose, two achieved a complete response (CR) and one a PR and proceeded to BMT. Three patients had progressive disease. Five of the six patients at the 40 mg/m(2) achieved a CR. Four proceeded to HSCT, and one relapsed prior to HSCT. One patient died on day 45 with marrow hypoplasia without evidence of leukemia. Hematologic and infectious adverse events were universal. The one dose limiting non-infectious toxicity observed was prolonged marrow hypoplasia. CONCLUSION: TVTC has significant anti-leukemic activity in both acute lymphoblastic and myeloblastic leukemia. The MTD of clofarabine is 40 mg/m(2)/day in this combination. This is the recommended dose for the phase II study in patients with refractory or relapsed leukemia, a population which has limited therapeutic options.

18. Sox2 transduction enhances cardiovascular repair capacity of blood-derived mesoangioblasts.

Circ Res. 2010 Apr 16;106(7):1290-302. Epub 2010 Feb 25.

Koyanagi M, Iwasaki M, Rupp S, Tedesco FS, Yoon CH, Boeckel JN, Trauth J, Schütz C, Ohtani K, Goetz R, Iekushi K, Bushoven P, Momma S, Mummery C, Passier R, Henschler R, Akintuerk H, Schranz D, Urbich C, Galvez BG, Cossu G, Zeiher AM, Dimmeler S.

RATIONALE: Complementation of pluripotency genes may improve adult stem cell functions.

OBJECTIVES: Here we show that clonally expandable, telomerase expressing progenitor cells can be isolated from peripheral blood of children. The surface marker profile of the clonally expanded cells is distinct from hematopoietic or mesenchymal stromal cells, and resembles that of embryonic multipotent mesoangioblasts. Cell numbers and proliferative capacity correlated with donor age. Isolated circulating mesoangioblasts (cMABs) express the pluripotency markers Klf4, c-Myc, as well as low levels of Oct3/4, but lack Sox2. Therefore, we tested whether overexpression of Sox2 enhances pluripotency and facilitates differentiation of cMABs in cardiovascular lineages.

METHODS AND RESULTS: Lentiviral transduction of Sox2 (Sox-MABs) enhanced the capacity of cMABs to differentiate into endothelial cells and cardiomyocytes in vitro. Furthermore, the number of smooth muscle actin positive cells was higher in Sox-MABs. In addition, pluripotency of Sox-MABs was shown by demonstrating the generation of endodermal and ectodermal progenies. To test whether Sox-MABs may exhibit improved therapeutic potential, we injected Sox-MABs into nude mice after acute myocardial infarction. Four weeks after cell therapy with Sox-MABs, cardiac function was significantly improved compared to mice treated with control cMABs. Furthermore, cell therapy with Sox-MABs resulted in increased number of differentiated cardiomyocytes, endothelial cells, and smooth muscle cells in vivo.

CONCLUSIONS: The complementation of Sox2 in Oct3/4-, Klf4-, and c-Myc-expressing cMABs enhanced the differentiation into all 3 cardiovascular lineages and improved the functional recovery after acute myocardial infarction.

19. Transplantation of reprogrammed embryonic stem cells improves visual function in a mouse model for retinitis pigmentosa.

Transplantation. 2010 Apr 27;89(8):911-9.

Wang NK, Tosi J, Kasanuki JM, Chou CL, Kong J, Parmalee N, Wert KJ, Allikmets R, Lai CC, Chien CL, Nagasaki T, Lin CS, Tsang SH.

BACKGROUND: To study whether C57BL/6J-Tyr/J (C2J) mouse embryonic stem (ES) cells can differentiate into retinal pigment epithelial (RPE) cells in vitro and then restore retinal function in a model for retinitis pigmentosa: Rpe65/Rpe65 C57BL6 mice.

METHODS: Yellow fluorescent protein (YFP)-labeled C2J ES cells were induced to differentiate into RPE-like structures on PA6 feeders. RPE-specific markers are expressed from differentiated cells in vitro. After differentiation, ES cell-derived RPE-like cells were transplanted into the subretinal space of postnatal day 5 Rpe65/Rpe65 mice. Live imaging of YFP-labeled C2J ES cells demonstrated survival of the graft. Electroretinograms (ERGs) were performed on transplanted mice to evaluate the functional outcome of transplantation.

RESULTS: RPE-like cells derived from ES cells sequentially express multiple RPE-specific markers. After transplantation, YFP-labeled cells can be tracked with live imaging for as long as 7 months. Although more than half of the mice were complicated with retinal detachments or tumor development, one fourth of the mice showed increased electroretinogram responses in the transplanted eyes. Rpe65/Rpe65 mice transplanted with RPE-like cells showed significant visual recovery during a 7-month period, whereas those injected with saline, PA6 feeders, or undifferentiated ES cells showed no rescue.

CONCLUSIONS: ES cells can differentiate, morphologically, and functionally, into RPE-like cells. Based on these findings, differentiated ES cells have the potential for the development of new therapeutic approaches for RPE-specific diseases such as certain forms of retinitis pigmentosa and macular degeneration. Nevertheless, stringent control of retinal detachment and teratoma development will be necessary before initiation of treatment trials.

20. Effect of carbamylated erythropoietin on major histocompatibility complex expression and neural differentiation of human neural stem cells.

J Neuroimmunol. 2010 Apr 15;221(1-2):15-24. Epub 2010 Feb 16.

Fu ZQ, Shao QL, Shen JL, Zhang YJ, Zhao XX, Yao L.

The expression of major histocompatibility complex (MHC) on human neural stem cells (hNSCs) is tightly related to the fate of these cells in transplantation, therefore strategies to relieve rejection and promote graft survival are necessary to be applied. This study investigated the effect of carbamylated erythropoietin (CEPO) on MHC expression and differentiation of hNSCs with or without IFN-gamma incubation. Results showed that low levels of MHC molecules were expressed on hNSCs and increased by IFN-gamma. CEPO enhanced MHC-I antigens in both proliferative and differentiated hNSCs, but decreased MHC-II antigens in differentiated hNSCs and those cells exposed to IFN-gamma. Furthermore, CEPO promoted neural differentiation of hNSCs and outgrowth of neurites. Western blot analysis revealed activation of Stat3, Stat5 and Akt during these processes. These results suggest that CEPO may have immunoregulatory function in hNSCs besides its neuroprotection.

21. Allo-HLA reactivity of virus-specific memory T cells is common.

Blood. 2010 Apr 15;115(15):3146-57. Epub 2010 Feb 16.

Amir AL, D'Orsogna LJ, Roelen DL, van Loenen MM, Hagedoorn RS, de Boer R, van der Hoorn MA, Kester MG, Doxiadis II, Falkenburg JH, Claas FH, Heemskerk MH.

Graft-versus-host disease and graft rejection are major complications of allogeneic HLA-mismatched stem cell transplantation or organ transplantation that are caused by alloreactive T cells. Because a range of acute viral infections have been linked to initiating these complications, we hypothesized that the cross-reactive potential of virus-specific memory T cells to allogeneic (allo) HLA molecules may be able to mediate these complications. To analyze the allo-HLA reactivity, T cells specific for Epstein-Barr virus, cytomegalovirus, varicella zoster virus, and influenza virus were tested against a panel of HLA-typed target cells, and target cells transduced with single HLA molecules. Eighty percent of T-cell lines and 45% of virus-specific T-cell clones were shown to cross-react against allo-HLA molecules. The cross-reactivity of the CD8 and CD4 T-cell clones was directed primarily against HLA class I and II, respectively. However, a restricted number of CD8 T cells exhibited cross-reactivity to HLA class II. T-cell receptor (TCR) gene transfer confirmed that allo-HLA reactivity and virus specificity were mediated via the same TCR. These results demonstrate that a substantial proportion of virus-specific T cells exert allo-HLA reactivity, which may have important clinical implications in transplantation settings as well as adoptive transfer of third-party virus-specific T cells.

22. Maintaining the male germline: regulation of spermatogonial stem cells.

J Endocrinol. 2010 May;205(2):133-45. Epub 2010 Feb 10.

Caires K, Broady J, McLean D.
Spermatogonial stem cells (SSCs) are a self-renewing population of adult stem cells capable of producing progeny cells for sperm production throughout the life of the male. Regulation of the SSC population includes establishment and maintenance of a niche microenvironment in the seminiferous tubules of the testis. Signaling from somatic cells within the niche determines the fate of SSCs by either supporting self-renewal or initiating differentiation leading to meiotic entry and production of spermatozoa. Despite the importance of these processes, little is known about the biochemical and cellular mechanisms that govern SSC fate and identity. This review discusses research findings regarding systemic, endocrine, and local cues that stimulate somatic niche cells to produce factors that contribute to the homeostasis of SSCs in mammals. In addition to their importance for male fertility, SSCs represent a model for the investigation of adult stem cells because they can be maintained in culture, and the presence, proliferation, or loss of SSCs in a cell population can be determined with the use of a transplantation assay. Defining the mechanisms that regulate the self-renewal and differentiation of SSCs will fundamentally improve the understanding of male fertility and provide information about the regulation of adult stem cells in other tissues.

23. Prognostic role of PET scanning before and after reduced-intensity allogeneic stem cell transplantation for lymphoma.

Blood. 2010 Apr 8;115(14):2763-8. Epub 2010 Feb 2.

Lambert JR, Bomanji JB, Peggs KS, Thomson KJ, Chakraverty RK, Fielding AK, Kottaridis PD, Roughton M, Morris EC, Goldstone AH, Linch DC, Ell PJ, Mackinnon S.

Allogeneic stem cell transplantation (SCT) is an established therapy for patients with relapsed lymphoma, but the role of positron emission tomography (PET) scanning preallogeneic and postallogeneic SCT is uncertain. We investigated whether pretransplantation PET status predicted outcome after allogeneic SCT and whether PET surveillance after transplantation provided additional information compared with computed tomography (CT) scanning. Eighty consecutive patients with lymphoma who received a reduced-intensity allogeneic SCT were entered onto a prospective trial. PET and CT scans were performed before transplantation and up to 36 months after transplantation. Forty-two patients were PET-positive before transplantation. Pretransplantation PET status had no significant impact on either relapse rate or overall survival. Thirty-four relapses were observed, of which 17 were PET-positive with a normal CT scan at relapse. Donor lymphocyte infusion (DLI) was administered in 26 episodes of relapse and was guided by PET alone in 14 patients. These findings suggest that, in contrast to autologous SCT, pretransplantation PET status is not predictive of relapse and survival after allogeneic SCT for lymphoma. Posttransplantation surveillance by PET detected relapse before CT in half of episodes, often allowing earlier administration of DLI in patients with recurrent lymphoma, and permitted withholding of potentially harmful DLI in those with PET-negative masses on CT scans.

24. Relapse of leukemia with loss of mismatched HLA resulting from uniparental disomy after haploidentical hematopoietic stem cell transplantation.

Blood. 2010 Apr 15;115(15):3158-61. Epub 2010 Feb 1.

Villalobos IB, Takahashi Y, Akatsuka Y, Muramatsu H, Nishio N, Hama A, Yagasaki H, Saji H, Kato M, Ogawa S, Kojima S.

We investigated human leukocyte antigen (HLA) expression on leukemic cells derived from patients at diagnosis and relapse after hematopoietic stem cell transplantation (HSCT) using flow cytometry with locus-specific antibodies. Two of 3 patients who relapsed after HLA-haploidentical HSCT demonstrated loss of HLA alleles in leukemic cells at relapse; on the other hand, no loss of HLA alleles was seen in 6 patients who relapsed after HLA-identical HSCT. Single-nucleotide polymorphism array analyses of sorted leukemic cells further revealed the copy number-neutral loss of heterozygosity, namely, acquired uniparental disomy on the short arm of chromosome 6, resulting in the total loss of the mismatched HLA haplotype. These results suggest that the escape from immunosurveillance by the loss of mismatched HLA alleles may be a crucial mechanism of relapse after HLA-haploidentical HSCT. Accordingly, the status of mismatched HLA on relapsed leukemic cells should be checked before donor lymphocyte infusion.

25. Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation.

Blood. 2010 Apr 15;115(15):3162-5. Epub 2010 Feb 1.

Venstrom JM, Gooley TA, Spellman S, Pring J, Malkki M, Dupont B, Petersdorf E, Hsu KC.

The natural killer cell receptor KIR3DS1 is associated with improved outcome in malignancies, infections, and autoimmune diseases, but data for the impact of KIR3DS1 in HSCT are inconsistent. Using genomic DNA from the National Marrow Donor Program, we performed donor KIR genotyping for 1087 patients who received an unrelated hematopoietic stem cell transplantation. A total of 33% of donors were KIR3DS1(+). Compared with KIR3DS1(-) donors, donor KIR3DS1 was associated with lower-grade II-IV acute graft-versus-host disease (GVHD; odds ratio = 0.71; 95% confidence interval, 0.55-0.92; P = .009), but not with relapse (hazard ratio = 0.97; 95% confidence interval, 0.73-1.29; P = .82). Furthermore, grade II-IV acute GVHD, overall mortality, and transplantation-related mortality all decreased as the number of copies of donor KIR3DS1 increased (P = .007, P = .03, and P = .02, respectively), with the lowest failure rate occurring among patients homozygous for donor KIR3DS1. Selection of donors with KIR3DS1 may decrease acute GVHD without compromising relapse-free

survival, separating the graft-versus-tumor effect from unwanted GVHD.

26. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells.

Blood. 2010 Apr 8;115(14):2960-70. Epub 2010 Jan 26.

Moosmann A, Bigalke I, Tischer J, Schirrmann L, Kasten J, Tippmer S, Leeping M, Prevalsek D, Jaeger G, Ledderose G, Mautner J, Hammerschmidt W, Schendel DJ, Kolb HJ.

Posttransplantation lymphoproliferative disease (PTLD) associated with Epstein-Barr virus (EBV) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation. PTLD is efficiently prevented by adoptive transfer of EBV-specific T cells from the donor. To make EBV-specific T cells available in urgent clinical situations, we developed a rapid protocol for their isolation by overnight stimulation of donor blood cells with peptides derived from 11 EBV antigens, interferon-gamma surface capture, and immunomagnetic separation. Six patients with PTLD received 1 transfusion of EBV-specific T cells. No response was seen in 3 patients who had late-stage disease with multiorgan dysfunction at the time of T-cell transfer. In 3 patients who received T cells at an earlier stage of disease, we observed complete and stable remission of PTLD. Two patients have remained free from EBV-associated disease for more than 2 years. CD8(+) T cells specific for EBV early antigens rapidly expanded after T-cell transfer, temporarily constituted greater than 20% of all peripheral blood lymphocytes, and were maintained throughout the observation period. Thus, a rapid and sustained reconstitution of a protective EBV-specific T-cell memory occurred after the infusion of small numbers of directly isolated EBV-specific T cells.

27. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells.

Blood. 2010 Apr 8;115(14):2769-76. Epub 2010 Jan 11.

Grigoriadis AE, Kennedy M, Bozec A, Brunton F, Stenbeck G, Park IH, Wagner EF, Keller GM.

The directed differentiation of human pluripotent stem cells offers the unique opportunity to generate a broad spectrum of human cell types and tissues for transplantation, drug discovery, and studying disease mechanisms. Here, we report the stepwise generation of bone-resorbing osteoclasts from human embryonic and induced pluripotent stem cells. Generation of a primitive streak-like population in embryoid bodies, followed by specification to hematopoiesis and myelopoiesis by vascular endothelial growth factor and hematopoietic cytokines in serum-free media, yielded a precursor population enriched for cells expressing the monocyte-macrophage lineage markers CD14, CD18, CD11b, and CD115. When plated in monolayer culture in the presence of macrophage colony-stimulating factor and receptor activator of nuclear factor-kappaB ligand (RANKL), these precursors formed large, multinucleated osteoclasts that expressed tartrate-resistant acid phosphatase and were capable of resorption. No tartrate-resistant acid phosphatase-positive multinucleated cells or resorption pits were observed in the absence of RANKL. Molecular analyses confirmed the expression of the osteoclast marker genes NFATc1, cathepsin K, and calcitonin receptor in a RANKL-dependent manner, and confocal microscopy demonstrated the coexpression of the alphavbeta3 integrin, cathepsin K and F-actin rings characteristic of active osteoclasts. Generating hematopoietic and osteoclast populations from human embryonic and induced pluripotent stem cells will be invaluable for understanding embryonic bone development and postnatal bone disease.

28. Delayed administration of filgrastim (G-CSF) following autologous peripheral blood stem cell transplantation (APBSCT) in pediatric patients does not change time to neutrophil engraftment and reduces use of G-CSF.

Pediatr Blood Cancer. 2010 May;54(5):728-33.

Pai V, Fernandez SA, Laudick M, Rosselet R, Termuhlen A.

BACKGROUND: Delayed initiation of granulocyte colony stimulating factor (G-CSF) after high-dose chemotherapy and autologous bone marrow or peripheral blood stem cell (APBSCT) in adult patients does not affect time to neutrophil or platelet engraftment, duration of fever, incidence of bacteremia, duration of non-

prophylactic antibiotic therapy, and length of hospitalization when compared to early initiation. This study compares the effect of delayed (day +6) versus early (day +1) administration of G-CSF in pediatric patients on time to neutrophil engraftment (TNE), duration and cost of G-CSF therapy, incidence of blood stream infections, duration of febrile-neutropenia, duration of non-prophylactic antibiotic therapy, and duration of hospitalization due to febrile-neutropenia. METHODS: This is a retrospective review of 65 patients who engrafted after receiving APBSCT and G-CSF between 1993 and 2006. They were divided into the delayed group (day +6) (n = 46) and the early group (day +1) (n = 19). RESULTS: The median ages were 4.7 and 5.3 years in the early and delayed groups, respectively. There was no significant difference in TNE (P = 0.06) between the two groups. The duration of G-CSF administration was significantly less in the delayed group (P = 0.003). No significant differences were observed in the duration of neutropenia, time to platelet engraftment, the incidence of blood stream infections, and duration of fevers. Duration of hospitalization due to febrile-neutropenia was significantly lower in the delayed group (P = 0.01). Significant cost savings were observed by delaying G-CSF administration. CONCLUSION: Delayed administration of G-CSF after APBSCT in children has no adverse effect on TNE or other clinical outcomes when compared to early administration and may incur substantial cost savings.

29. Shedding of the endothelial receptor tyrosine kinase Tie2 correlates with leukemic blast burden and outcome after allogeneic hematopoietic stem cell transplantation for AML.

Ann Hematol. 2010 May;89(5):459-67.

Koenecke C, Kümpers P, Lukasz A, Dammann E, Verhagen W, Göhring G, Buchholz S, Krauter J, Eder M, Schlegelberger B, Ganser A.

Angiogenesis plays an important role in the growth and viability of hematologic malignancies. Emerging data suggest a crucial involvement of the endothelial-specific Tie2 receptor and its antagonistic ligand Angiopoietin-2 (Ang-2) in this process. The purpose of this study was to elucidate whether the soluble domain of the Tie2 receptor (sTie2)predicts outcome in patients with acute myeloid leukemia(AML) undergoing allogeneic hematopoietic stem cell transplantation(HSCT). Serum levels of sTie2 and Ang-2 were measured by ELISA in 181 AML patients before conditioning for HSCT. The median follow-up time was 22 months after HSCT. Pre-HSCT sTie2 levels were significantly higher inpatients (median 2.2 (range 1.8-3.0) ng/mL) compared to healthy controls (1.3 (0.9-1.6); p<0.0001). Elevated sTie2 levels were independently associated with active AML but did not relate to cytogenetics/mutational status before transplantation. Logistic regression analysis identified elevated sTie2 (odds ratio (OR) 3.07 (95% confidence interval(CI; 1.56-6.04), p=0.001) as a strong predictor for disease relapse and poor overall survival after HSCT. In a multimarker approach the highest risk for relapse was observed inpatients with both elevated sTie2 and elevated Ang-2 (OR 4.07, (95% CI 1.79-9.25) p<0.0001), as well as patients with both elevated Ang-2 and elevated bone marrow blast count (OR 4.16, (95% CI 1.88-7.36) p<0.0001). Elevated serum sTie2 levels were related to active leukemia,correlated with the percentage of leukemic blasts in the bone marrow, and independently predicted relapse in AML patients after allogeneic HSCT. Furthermore, our data indicate that Tie2 shedding and Ang-2 release seem to reflect overlapping, but nevertheless distinctive features in leukemia-associated neoangiogenesis.

30. Severe ehrlichia infection in pediatric oncology and stem cell transplant patients.

Pediatr Blood Cancer. 2010 May;54(5):776-8.

Esbenshade A, Esbenshade J, Domm J, Williams J, Frangoul H.

Ehrlichiosis, a tickborne illness transmitted by tick vectors Amblyomma americanum and Ixodes scapularis, can be acquired in endemic areas. Clinical manifestations range from asymptomatic to fulminant in nature. We report three cases of ehrlichiosis in pediatric oncology patients, one of whom was a stem cell transplant recipient. Early symptoms included fever, malaise, and vague gastrointestinal symptoms. Laboratory abnormalities were initially attributed to chemotherapy toxicity. Illness was severe in all three patients and one patient died even after initiation of doxycycline. These cases emphasize the need for a high index of suspicion for tickborne illness in oncology patients, and the importance of a low threshold for starting empiric treatment before confirming the diagnosis.

31. Dendritic cells are susceptible to infection with wild-type adenovirus, inducing a differentiation arrest in precursor cells and inducing a strong T-cell stimulation.

J Gen Virol. 2010 May;91(Pt 5):1150-4. Epub 2009 Dec 23.

Kessler T, Hamprecht K, Feuchtinger T, Jahn G.

Adenovirus infection after stem cell transplantation is a significant cause of morbidity and mortality, especially in children. A robust T-cell response induced by dendritic cells (DC) is crucial for clearing the virus, suggesting their pivotal role for the response to human adenoviruses (HAdV). Despite the widespread use of adenoviral vectors, the properties and kinetics of HAdV infection of DC have not been addressed yet. We show that a recent clinical HAdV, subgenus C/serotype 2 (strain BB2000-61), infects cells of the myeloid lineage. Infected DC produce early and late viral antigens and show an altered expression of surface markers. Infection of monocytes renders them refractory to differentiation into DC. Additionally, HAdV-infected DC are strong stimulators of CD8+ T cells. In summary, HAdV seems to manipulate the immune response by infection of DC and possibly uses the infection of monocytes as a means to escape recognition by T cells.

32. Bortezomib, thalidomide, dexamethasone induction therapy followed by melphalan, prednisolone, thalidomide consolidation therapy as a first line of treatment for patients with multiple myeloma who are non-transplant candidates: results of the Korean Multiple Myeloma Working Party (KMMWP).

Ann Hematol. 2010 May;89(5):489-97. Epub 2009 Dec 10.

Eom HS, Kim YK, Chung JS, Kim K, Kim HJ, Kim HY, Jin JY, Do YR, Oh SJ, Suh C, Seong CM, Kim CS, Lee DS, Lee JH.

Bortezomib (VELCADE), thalidomide and dexamethasone (VTD), as well as melphalan, prednisolone, and thalidomide (MPT) therapy, are highly effective in patients with multiple myeloma. We evaluated the responses and survival times of 35 patients treated with VTD followed by MPT. All patients were newly diagnosed and non-transplantation candidates. Patients received six cycles of VTD, which were followed by eight cycles of MPT. Approximately 97% of patients exhibited early responses to therapy, as early as the second cycle of VTD. Thirty percent of the responses were high quality, which was defined as a complete response (CR), a near-CR or a very good partial response. High-risk patients were defined as patients with any of the following aberrations: del(13), t(4;14), or del(17p). The remaining patients were defined as standard risk. Eleven high-risk patients showed 100% response rates, including 91% high-quality responses. In contrast, 13 standard-risk patients exhibited 92% response rates, including 61% high-quality responses. The overall 2-year survival rates were 60% in high-risk patients and 85% in standard-risk patients, which was not significantly different. As a first-line therapy, VTD followed by MPT has the potential to provide high-quality responses with durable remission among elderly and high-risk patients (clinicaltrials.gov identifier: NCT00320476).

33. The sumoylation pathway is dysregulated in multiple myeloma and is associated with adverse patient outcome.

Blood. 2010 Apr 8;115(14):2827-34. Epub 2009 Nov 30.

Driscoll JJ, Pelluru D, Lefkimmiatis K, Fulciniti M, Prabhala RH, Greipp PR, Barlogie B, Tai YT, Anderson KC, Shaughnessy JD Jr, Annunziata CM, Munshi NC.

Multiple myeloma (MM) is a plasma cell neoplasm that proceeds through a premalignant state of monoclonal gammopathy of unknown significance; however, the molecular events responsible for myelomagenesis remain uncharacterized. To identify cellular pathways deregulated in MM, we addressed that sumoylation is homologous to ubiquitination and results in the attachment of the ubiquitin-like protein Sumo onto target proteins. Sumoylation was markedly enhanced in MM patient lysates compared with normal plasma cells and expression profiling indicated a relative induction of sumoylation pathway genes. The Sumo-conjugating enzyme Ube2I, the Sumo-ligase PIAS1, and the Sumo-inducer ARF were elevated in MM patient samples and cell lines. Survival correlated with expression because 80% of patients with low UBE2I and PIAS1 were living 6 years after transplantation, whereas only 45% of patients with high expression survived 6 years. UBE2I encodes the sole Sumo-conjugating enzyme in mammalian cells and cells transfected with a dominant-negative sumoylation-deficient UBE2I mutant exhibited decreased survival after radiation exposure, impaired adhesion to bone marrow stroma cell and decreased bone marrow stroma cell-induced proliferation. UBE2I confers cells with

multiple advantages to promote tumorigenesis and predicts decreased survival when combined with PIAS1. The sumoylation pathway is a novel therapeutic target with implications for existing proteasomal-based treatment strategies.

34. Human nucleus pulposus cells significantly enhanced biological properties in a coculture system with direct cell-to-cell contact with autologous mesenchymal stem cells.

J Orthop Res. 2010 May;28(5):623-30.

Watanabe T, Sakai D, Yamamoto Y, Iwashina T, Serigano K, Tamura F, Mochida J.

Activated nucleus pulposus (NP) cells can be reinserted into the disc to inhibit intervertebral disc degeneration. Experimental studies in animals showed that using a coculture system with direct cell-to-cell contact with mesenchymal stem cells (MSCs) significantly upregulated the biological activity of NP cells. The purpose of this study is to determine whether this activation of NP cells by autologous MSCs is applicable to human cells in vitro. Human NP tissue was obtained from surgical specimens and MSCs from bone marrow of 10 subjects. Six-well culture plates and inserts were used for culture; 1.0x10(4) NP cells were seeded onto each insert and incubated alone, in standard coculture with 1.0x10(4) MSCs, or cocultured with direct cell-to-cell contact. NP cell proliferation, DNA synthesis, and proteoglycan (PG) synthesis were evaluated. Chromosome abnormalities in the activated NP cells and tumorigenesis of the cells were evaluated in an additional 10 patients by microscopic examination for segmented cells and histological assessment of activated cells transplanted into nude mice. Cell proliferation, DNA synthesis, and PG synthesis were significantly upregulated. The positive effects of the coculture system with direct cell-to-cell contact seen in animal studies were also confirmed in human cells. Chromosome abnormalities and tumorigenesis were not observed in the activated NP cells. In conclusion, a coculture system with direct cell-to-cell contact demonstrated a significant positive effect, enhancing the biological properties of human NP cells, as it did in animal models. These results should prove useful for conducting trials leading to the clinical use of activated NP cell transplantation.

35. Salvage treatment with upfront melphalan 100 mg/m(2) and consolidation with novel drugs for fulminant progression of multiple myeloma.

Ann Hematol. 2010 May;89(5):483-7. Epub 2009 Nov 19.

Krejci M, Adam Z, Buchler T, Krivanova A, Pour L, Zahradova L, Holanek M, Sandecka V, Mayer J, Vorlicek J, Hajek R.

Patients (pts) with fulminant progression (FPG) of multiple myeloma (MM) after autologous stem cell transplantation (ASCT) have poor prognosis. Pancytopenia, extramedullary disease, and/or renal impairment are often present, and treatment options are limited. We have retrospectively evaluated 31 pts with FPG of MM after ASCT who were treated upfront salvage therapy with melphalan 100 mg/m(2) (MEL 100) followed by PBSC support and consolidation therapy using regimens containing thalidomide (n = 16) or bortezomib (n = 15). The overall response rate (ORR) was 58% (18/31). After MEL 100, one patient achieved complete remission (3%), 26% of pts very good partial remission, 29% of pts partial remission, and 42% of pts stable disease. Progression within 3 months after MEL 100 occurred in 35% of pts. The median follow-up from MEL 100 was 8 months. The median TTP was 5 months (range, 2-15 months), and the median OS was 8 months (range, 3-23 months). There were no treatment-related deaths. In fulminant progression of MM, upfront MEL 100 is a safe salvage regimen with good response rate (ORR, 58%). Treatment with upfront MEL 100 followed by a thalidomide- or bortezomib-based regimen can prolong overall survival to more than 12 months in 33% of pts with fulminant progression of MM.

36. Improved T and B cell recovery by the transfer of slowly dividing human hematopoietic stem cells.

Leuk Res. 2010 May;34(5):622-30. Epub 2009 Nov 10. Comment in: Leuk Res. 2010 May;34(5):572-3.

Vitacolonna M, Schubert M, Herbert N, Taubert I, Singh R, Ho A, Zöller M.

Human hematopoietic stem cells giving rise to long term initiating cells in vitro are enriched in a CD34(+) slow

dividing fraction (SDF). Here, we tested reconstitution and multilineage differentiation of this CD34(+) SDF in NOD/SCID mice. In the bone marrow a slightly higher percentage of human hematopoietic progenitors were recovered after the transfer of the SDF compared to the fast dividing fraction. Instead, T cell maturation in the rudimentary thymus and lymph node repopulation was only initiated by the SDF. The capacity of the SDF to differentiate and mature in the patients' thymus could provide an advantage in immunocompetence recovery.

37. Hepato-biliary late effects in survivors of childhood and adolescent cancer: a report from the Children's Oncology Group.

Pediatr Blood Cancer. 2010 May;54(5):663-9.

Castellino S, Muir A, Shah A, Shope S, McMullen K, Ruble K, Barber A, Davidoff A, Hudson MM.

Curative therapy for childhood and adolescent cancer translates to 1 in 640 young adults being a survivor of cancer. Although acute hepato-biliary toxicity occurs commonly during pediatric cancer therapy, the impact of antineoplastic therapy on long-term liver health in childhood/adolescent cancer survivors is unknown. This article reviews the medical literature on late liver dysfunction following treatment for childhood/adolescent cancer. We also outline the Children's Oncology Group (COG) guidelines for screening and follow-up of hepato-biliary sequelae. As the population of survivors grow and age, vigilance for risks to hepatic health needs to continue based on specific exposures during curative cancer therapy.

38. Impact of critical care reconfiguration and track-and-trigger outreach team intervention on outcomes of haematology patients requiring intensive care admission.

Ann Hematol. 2010 May;89(5):505-12. Epub 2009 Oct 30.

Bokhari SW, Munir T, Memon S, Byrne JL, Russell NH, Beed M.

Patients with haematological disorders have previously been considered to have poor outcomes following admission to intensive care units. Although a number of haematology centres from outside the UK have now demonstrated improved outcomes, the continuing perception of poor outcomes in this patient group continues to adversely affect their chances of being admitted to some intensive care units (ICUs). Over the past 10 years, there have been many advances within the disciplines of both haematology and intensive care medicine. This study was done to assess outcomes and the impact of an early warning scoring system (EWS) and early involvement of ICU outreach teams. One hundred five haematology patients (haematopoietic stem cell transplant (HSCT) or non-HSCT) had 114 admissions to ICU between April 2006 and August 2008 which coincided with hospital-wide implementation of EWS. The survival to ICU discharge was 56 (53%). Thirty-three (33%) patients were alive at 6 months giving a 1-year survival of 31%. Of the 39 HSCT patients, nine were post-autologous and 30 post-allogeneic transplant. The survival to ICU discharge was 22 (56%) with 14 (36%) patients alive at 6 months. One year survival was 36%. Prior to the introduction of EWS and critical care outreach team (2004), survival to ICU discharge was 44% which has increased to 53% (2006-2008). This is despite an increase in mechanical ventilation in 2006-2008 (50%) as compared to 2004 (32%).The improvement in ICU survivorship was even more prominent in HSCT patients (37% in 2004 versus 56% in 2006-2008). There was a trend towards decreasing Acute Physiology and Chronic Health Evaluation scores with time, suggesting appropriate patients being identified earlier and having timely escalation of their treatment.

39. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia.

Blood. 2010 Apr 8;115(14):2872-81. Epub 2009 Oct 27. Comment in: Blood. 2010 Apr 8;115(14):2726-7.

Nakahara F, Sakata-Yanagimoto M, Komeno Y, Kato N, Uchida T, Haraguchi K, Kumano K, Harada Y, Harada H, Kitaura J, Ogawa S, Kurokawa M, Kitamura T, Chiba S.

Hairy enhancer of split 1 (Hes1) is a basic helix-loop-helix transcriptional repressor that affects differentiation and often helps maintain cells in an immature state in various tissues. Here we show that retroviral expression of Hes1 immortalizes common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs) in the presence of interleukin-3, conferring permanent replating capability on these cells. Whereas these cells did

not develop myeloproliferative neoplasms when intravenously administered to irradiated mice, the combination of Hes1 and BCR-ABL in CMPs and GMPs caused acute leukemia resembling blast crisis of chronic myelogenous leukemia (CML), resulting in rapid death of the recipient mice. On the other hand, BCR-ABL alone caused CML-like disease when expressed in c-Kit-positive, Sca-1-positive, and lineage-negative hematopoietic stem cells (KSLs), but not committed progenitors CMPs or GMPs, as previously reported. Leukemic cells derived from Hes1 and BCR-ABL-expressing CMPs and GMPs were more immature than those derived from BCR-ABL-expressing KSLs. Intriguingly, Hes1 was highly expressed in 8 of 20 patients with CML in blast crisis, but not in the chronic phase, and dominant negative Hes1 retarded the growth of some CML cell lines expressing Hes1. These results suggest that Hes1 is a key molecule in blast crisis transition in CML.

40. Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma.

Cancer Lett. 2010 Apr 28;290(2):157-66. Epub 2009 Sep 27.

Gao P, Ding Q, Wu Z, Jiang H, Fang Z.

Mesenchymal stem cells (MSCs) represent a new tool for delivery of therapeutic agents to cancer. The cytokine interleukin-12 (IL-12) has demonstrated a potent anti-tumor activity in a variety of mouse tumor models. In this study, human MSCs were isolated from human bone marrow and identified by phenotype analysis and differentiation assays. The anti-tumor activity of human MSCs stably transduced with a recombinant adenoviral vector expressing the murine IL-12 (MSC/IL-12) were evaluated in a mouse xenograft model of renal cell carcinoma (RCC). Expression and bioactivity of the transgenic protein IL-12 from adenoviral vector were confirmed prior to in vivo studies. A nude mouse model of RCC was developed by subcutaneously injection of 786-0 cells into nude mice. MSC/IL-12 was injected into the lateral tail vein with single dose. Results indicated that systemic administration of MSC/IL-12 reduced the growth of 786-0 RCC and significantly prolonged mouse survival. These transfected cells could home to tumors after intravenous injection and largely produce local IL-12 protein. In contrast, systemic level of IL-12 was modestly elevated. Further studies showed that the anti-tumor activity of the MSC/IL-12 was dependent on the presence of natural killer (NK) cells and IFN-gamma in this experimental setting. These data demonstrate the potential of adult MSC constitutively producing IL-12 to reduce the growth of RCC and enhance the tumor-bearing mouse survival.

41. Hospicells (ascites-derived stromal cells) promote tumorigenicity and angiogenesis.

Int J Cancer. 2010 May 1;126(9):2090-101.

Pasquet M, Golzio M, Mery E, Rafii A, Benabbou N, Mirshahi P, Hennebelle I, Bourin P, Allal B, Teissie J, Mirshahi M, Couderc B.

The microenvironment is known to play a dominant role in cancer progression. Cells closely associated with tumoral cells, named hospicells, have been recently isolated from the ascites of ovarian cancer patients. Whilst these cells present no specific markers from known cell lineages, they do share some homology with bone marrow-derived or adipose tissue-derived human mesenchymal stem cells (CD9, CD10, CD29, CD146, CD166, HLA-1). We studied the role of hospicells in ovarian carcinoma progression. In vitro, these cells had no effect on the growth of human ovarian carcinoma cell lines OVCAR-3, SKOV-1 and IGROV-1. In vivo, their co-injection with adenocarcinoma cells enhanced tumor growth whatever the tumor model used (subcutaneous and intraperitoneally established xenografts in athymic mice). In addition, their injection increased the development of ascites in tumor-bearing mice. Fluorescent macroscopy revealed an association between hospicells and ovarian adenocarcinoma cells within the tumor mass. Tumors obtained by coinjection of hospicells and human ovarian adenocarcinoma cells presented an increased microvascularization indicating that the hospicells could promote tumorigenicity of ovarian tumor cells in vivovia their action on angiogenesis. This effect on angiogenesis could be attributed to the increased HIF1alpha and VEGF expression associated with the presence of the hospicells. Collectively, these data indicate a role for these ascite-derived stromal cells in promoting tumor growth by increasing angiogenesis.

42. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial.

Exp Neurol. 2010 May;223(1):229-37. Epub 2009 Aug 13.

Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K, Testa L, Stecco A, Tarletti R, Miglioretti M, Fava E, Nasuelli N, Cisari C, Massara M, Vercelli R, Oggioni GD, Carriero A, Cantello R, Monaco F, Fagioli F.

Amyotrophic Lateral Sclerosis (ALS) is a devastating incurable disease. Stem-cell-based therapies represent a new possible strategy for ALS clinical research. The objectives of this Phase 1 clinical study were to assess the feasibility and toxicity of mesenchymal stem cell transplantation and to test the impact of a cell therapy in ALS patients. The trial was approved and monitored by the National Institute of Health and by the Ethics Committees of all participating Institutions. Autologous MSCs were isolated from bone marrow, expanded in vitro and analyzed according to GMP conditions. Expanded MSCs were suspended in the autologous cerebrospinal fluid (CSF) and directly transplanted into the spinal cord at a high thoracic level with a surgical procedure. Ten ALS patients were enrolled and regularly monitored before and after transplantation by clinical, psychological, neuroradiological and neurophysiological assessments. There was no immediate or delayed transplant-related toxicity. Clinical, laboratory, and radiographic evaluations of the patients showed no serious transplant-related adverse events. Magnetic resonance images (MRI) showed no structural changes (including tumor formation) in either the brain or the spinal cord. However the lack of post mortem material prevents any definitive conclusion about the vitality of the MSCs after transplantation. In conclusion, this study confirms that MSC transplantation into the spinal cord of ALS patients is safe and that MSCs might have a clinical use for future ALS cell based clinical trials.

43. Involved field radiation after autologous stem cell transplant for diffuse large B-cell lymphoma in the rituximab era.

Int J Radiat Oncol Biol Phys. 2010 May 1;77(1):79-85. Epub 2009 Aug 3.

Biswas T, Dhakal S, Chen R, Hyrien O, Bernstein S, Friedberg JW, Fisher RI, Liesveld J, Phillips G, Constine LS.

PURPOSE: For patients with recurrent or refractory large B-cell non-Hodgkin's lymphoma, high-dose chemotherapy and autologous stem cell transplant (ASCT) is the treatment of choice. We evaluated the role of involved field radiation therapy (IFRT) post-ASCT for patients initially induced with cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP) or, more recently, rituximab-CHOP (R-CHOP).

MATERIALS AND METHODS: Between May 1992 and April 2005, 176 patients underwent ASCT for recurrent or refractory large B-cell non-Hodgkin's lymphoma; 164 patients were evaluable for endpoint analysis. Fifty percent of the CHOP group (n = 131), and 39% of the R-CHOP group (n = 33), received IFRT. Follow-up from the time of transplant was a median/mean of 1.7/3 years (range, 0.03-13 years).

RESULTS: The 5-year overall survival (OS) and disease-specific survival (DSS) improved with IFRT in both the R-CHOP (p = 0.006 and 0.02, respectively) and CHOP (p = 0.02 and p = 0.04, respectively) groups. IFRT was associated with a 10% (p = 0.17) reduction in local failure, alone or with a distant site. On univariate analysis, IFRT was associated with superior OS (hazard ratio [HR] = 0.50 [95% CI 0.32, 0.78]; p = 0.002) and DSS (HR = 0.53 [95% CI 0.33, 0.86]; p = 0.009). Presence of B symptoms was adverse (p = 0.03). On multivariate analysis, only IFRT was associated with significant improvement in OS (HR = 0.35 [0.18, 0.68]; p = 0.002) and DSS (HR = 0.39 [95% CI 0.18, 0.84]; p = 0.01).

CONCLUSIONS: Recognizing that positive and negative patient selection bias exists for the use of IFRT post-ASCT, patients initially treated with CHOP or R-CHOP and who undergo ASCT for recurrent or refractory disease may benefit from subsequent IFRT presumably due to enhanced local control that can translate into a survival advantage.

44. Skin-derived precursor cells enhance peripheral nerve regeneration following chronic denervation.

Exp Neurol. 2010 May;223(1):221-8. Epub 2009 May 27.

Walsh SK, Gordon T, Addas BM, Kemp SW, Midha R.

While peripheral nerves demonstrate the capacity for axonal regeneration, outcome following injury remains relatively poor, especially following prolonged denervation. Since axon-deprived Schwann cells (SCs) in the distal nerve progressively lose their ability to support axonal growth, we took the approach of using skin-derived precursor cells (SKPs) as an accessible source of replacement SCs that could be transplanted into chronically denervated peripheral nerve. In this study, we employed a delayed cross-reinnervation paradigm to assess regeneration of common peroneal nerve axons into the chronically denervated rodent tibial nerve following delivery of SKP-derived SC (SKP-SCs). SKP-SC treated animals exhibited superior axonal regeneration to media controls, with significantly higher counts of regenerated motorneurons and histological recovery similar to that of immediately repaired nerve. Improved axonal regeneration correlated with superior muscle reinnervation, as measured by compound muscle action potentials and wet muscle weights. We therefore conclude that SKPs represent an easily accessible, autologous source of stem cell-derived Schwann cells that show promise in improving regeneration through chronically injured nerves.

NITRIC OXIDE AND MUSCLE

1. Oxidative stress regulates left ventricular PDE5 expression in the failing heart.

Circulation. 2010 Apr 6;121(13):1474-83. Epub 2010 Mar 22.

Lu Z, Xu X, Hu X, Lee S, Traverse JH, Zhu G, Fassett J, Tao Y, Zhang P, dos Remedios C, Pritzker M, Hall JL, Garry DJ, Chen Y.

BACKGROUND: Phosphodiesterase type 5 (PDE5) inhibition has been shown to exert profound beneficial effects in the failing heart, suggesting a significant role for PDE5 in the development of congestive heart failure (CHF). The purpose of this study is to test the hypothesis that oxidative stress causes increased PDE5 expression in cardiac myocytes and that increased PDE5 contributes to the development of CHF.

METHODS AND RESULTS: Myocardial PDE5 expression and cellular distribution were determined in left ventricular samples from patients with end-stage CHF and normal donors and from mice after transverse aortic constriction (TAC)-induced CHF. Compared with donor human hearts, myocardial PDE5 protein was increased approximately equal 4.5-fold in CHF samples, and the increase of myocardial PDE5 expression was significantly correlated with myocardial oxidative stress markers 3'-nitrotyrosine or 4-hydroxynonenal expression ($P<0.05$). Histological examination demonstrated that PDE5 was mainly expressed in vascular smooth muscle in normal donor hearts, but its expression was increased in both cardiac myocytes and vascular smooth muscle of CHF hearts. Myocardial PDE5 protein content and activity also increased in mice after TAC-induced CHF ($P<0.05$). When the superoxide dismutase (SOD) mimetic M40401 was administered to attenuate oxidative stress, the increased PDE5 protein and activity caused by TAC was blunted, and the hearts were protected against left ventricular hypertrophy and CHF. Conversely, increased myocardial oxidative stress in superoxide dismutase 3 knockout mice caused a greater increase of PDE5 expression and CHF after TAC. In addition, administration of sildenafil to inhibit PDE5 attenuated TAC-induced myocardial oxidative stress, PDE5 expression, and CHF.

CONCLUSIONS: Myocardial oxidative stress increases PDE5 expression in the failing heart. Reducing oxidative stress by treatment with M40401 attenuated cardiomyocyte PDE5 expression. This and selective inhibition of PDE5 protected the heart against pressure overload-induced left ventricular hypertrophy and CHF.

2. Inotropic response of cardiac ventricular myocytes to beta-adrenergic stimulation with isoproterenol exhibits diurnal variation: involvement of nitric oxide.

Circ Res. 2010 Apr 16;106(7):1244-52. Epub 2010 Feb 18.

Collins HE, Rodrigo GC.

RATIONALE: Although >10% of cardiac gene expression displays diurnal variations, little is known of their impact on excitation-contraction coupling.

OBJECTIVE: To determine whether the time of day affects excitation-contraction coupling in rat ventricles.

METHODS AND RESULTS: Left ventricular myocytes were isolated from rat hearts at 2 opposing time points, corresponding to the animals resting or active periods. Basal contraction and [Ca(2+)](i) was significantly greater in myocytes isolated during the resting versus active periods (cell shortening 12.4+/-0.3 versus 11.0+/-0.2%; P<0.05 and systolic [Ca(2+)](i) 422+/-12 versus 341+/-9 nmol/L; P<0.01. This corresponded to a greater sarcoplasmic reticulum (SR) Ca(2+) load (672+/-20 versus 551+/-13 nmol/L P<0.001). The increase in systolic [Ca(2+)](i) in response to isoproterenol (>3 nmol/L) was also significantly greater in resting versus active period myocytes, reflecting a greater SR Ca(2+) load at this time. This diurnal variation in response of Ca(2+)-homeostasis to isoproterenol translated to a greater incidence of arrhythmic activity in resting period myocytes. Inhibition of neuronal NO synthase during stimulation with isoproterenol, further increased systolic [Ca(2+)](i) and the percentage of arrhythmic myocytes, but this effect was significantly greater in active period versus resting period myocytes. Quantitative RT-PCR analysis revealed a 2.65-fold increase in neuronal NO synthase mRNA levels in active over resting period myocytes (P<0.05).

CONCLUSIONS: The threshold for the development of arrhythmic activity in response to isoproterenol is higher during the active period of the rat. We suggest this reflects a reduction in SR Ca(2+) loading and a diurnal variation in neuronal NO synthase signaling.

HMGB

1. Overexpression of high-mobility group box 1 correlates with tumor progression and poor prognosis in human colorectal carcinoma.

J Cancer Res Clin Oncol. 2010 May;136(5):677-84. Epub 2009 Nov 7.

Yao X, Zhao G, Yang H, Hong X, Bie L, Liu G.

PURPOSE: High-mobility group box 1 (HMGB1) has been implicated in a variety of biologically important processes, including transcription, DNA repair, V(D)J recombination, differentiation, development, and extracellular signaling. The increased expression of HMGB1 has been described in colorectal cancer (CRC). However, there is no report about the correlation of HMGB1 with clinicopathologic features, including the survival of patients with CRC.

METHODS: In present study, we investigated HMGB1 expression and its prognostic significance in CRC by performing immunohistochemical analysis, using a total of 192 paraffin-embedded archival CRC samples. Moreover, disruption of endogenous HMGB1 protein through a siRNA knockdown technique was performed to investigate the possible role of HMGB1 in the process of tumor invasion and metastasis.

RESULTS: Overexpression of HMGB1 was shown in 55.7% cases. Statistical analysis showed that HMGB1 expression was positively correlated with tumor invasion (P = 0.048), lymph node metastasis (P = 0.011), distant metastasis (P = 0.031), and Duke's stage (P = 0.029) of CRC patients. Patients with higher HMGB1 expression had shorter overall survival time, whereas patients with lower level of HMGB1 had better survival. Multivariate analysis suggested that HMGB1 expression might be an independent prognostic indicator for the survival of patients with CRC. Disruption of endogenous HMGB1 protein through a siRNA knockdown technique was shown to suppress substantially the invasion ability of SW620 cells.

CONCLUSIONS: Our results suggest that HMGB1 protein is a valuable marker for progression of CRC patients. High HMGB1 expression is associated with poor overall survival in patients with CRC.

HDAC

1. Sp1/NFkappaB/HDAC/miR-29b regulatory network in KIT-driven myeloid leukemia.

Cancer Cell. 2010 Apr 13;17(4):333-47.

Liu S, Wu LC, Pang J, Santhanam R, Schwind S, Wu YZ, Hickey CJ, Yu J, Becker H, Maharry K, Radmacher MD, Li C, Whitman SP, Mishra A, Stauffer N, Eiring AM, Briesewitz R, Baiocchi RA, Chan KK, Paschka P, Caligiuri MA, Byrd JC, Croce CM, Bloomfield CD, Perrotti D, Garzon R, Marcucci G.

The biologic and clinical significance of KIT overexpression that associates with KIT gain-of-function mutations occurring in subsets of acute myeloid leukemia (AML) (i.e., core binding factor AML) is unknown. Here, we show that KIT mutations lead to MYC-dependent miR-29b repression and increased levels of the miR-29b target Sp1 in KIT-driven leukemia. Sp1 enhances its own expression by participating in a NFkappaB/HDAC complex that further represses miR-29b transcription. Upregulated Sp1 then binds NFkappaB and transactivates KIT. Therefore, activated KIT ultimately induces its own transcription. Our results provide evidence that the mechanisms of Sp1/NFkappaB/HDAC/miR-29b-dependent KIT overexpression contribute to leukemia growth and can be successfully targeted by pharmacological disruption of the Sp1/NFkappaB/HDAC complex or synthetic miR-29b treatment in KIT-driven AML.

2. Membrane-associated glucocorticoid activity is necessary for modulation of long-term memory via chromatin modification.

J Neurosci. 2010 Apr 7;30(14):5037-46.

Roozendaal B, Hernandez A, Cabrera SM, Hagewoud R, Malvaez M, Stefanko DP, Haettig J, Wood MA.

Glucocorticoid hormones enhance the consolidation of long-term memory of emotionally arousing training experiences. This memory enhancement requires activation of the cAMP-dependent kinase pathway and the subsequent phosphorylation of cAMP response-element binding (CREB) protein. Here, we demonstrate that glucocorticoids enhance the consolidation of hippocampus-dependent and hippocampus-independent aspects of object recognition memory via chromatin modification. More specifically, systemic corticosterone increases histone acetylation, a form of chromatin modification, in both the hippocampus and insular cortex following training on an object recognition task. This led us to examine whether increasing histone acetylation via histone deacetylase (HDAC) inhibition enhances memory in a manner similar to corticosterone. We found a double dissociation between posttraining HDAC inhibitor infusion into the insular cortex and hippocampus on the enhancement of object recognition and object location memory, respectively. In determining the molecular pathway upstream of glucocorticoids' effects on chromatin modification, we found that activation of membrane-associated glucocorticoid receptors (GRs) and the subsequent interaction between phospho-CREB and CREB-binding protein (CBP) appear to be necessary for glucocorticoids to enhance memory consolidation via chromatin modification. In contrast, mineralocorticoid receptors (MRs) do not appear to be involved. The findings also indicate that glucocorticoid activity has differential influences on hippocampus-dependent and hippocampus-independent components of memory for objects.

3. Histone deacetylase 7 controls endothelial cell growth through modulation of beta-catenin.

Circ Res. 2010 Apr 16;106(7):1202-11. Epub 2010 Mar 11. Comment in: Circ Res. 2010 Apr 16;106(7):1180-3.

Margariti A, Zampetaki A, Xiao Q, Zhou B, Karamariti E, Martin D, Yin X, Mayr M, Li H, Zhang Z, De Falco E, Hu Y, Cockerill G, Xu Q, Zeng L.

RATIONALE: Histone deacetylase (HDAC)7 is expressed in the early stages of embryonic development and may play a role in endothelial function.

OBJECTIVE: This study aimed to investigate the role of HDAC7 in endothelial cell (EC) proliferation and growth and the underlying mechanism.

METHODS AND RESULTS: Overexpression of HDAC7 by adenoviral gene transfer suppressed human umbilical vein endothelial cell (HUVEC) proliferation by preventing nuclear translocation of beta-catenin and downregulation of T-cell factor-1/Id2 (inhibitor of DNA binding 2) and cyclin D1, leading to G(1) phase elongation. Further assays with the TOPFLASH reporter and quantitative RT-PCR for other beta-catenin target genes such as Axin2 confirmed that overexpression of HDAC7 decreased beta-catenin activity. Knockdown of HDAC7 by lentiviral short hairpin RNA transfer induced beta-catenin nuclear translocation but downregulated cyclin D1, cyclin E1 and E2F2, causing HUVEC hypertrophy. Immunoprecipitation assay and mass spectrometry analysis

revealed that HDAC7 directly binds to beta-catenin and forms a complex with 14-3-3 epsilon, zeta, and eta proteins. Vascular endothelial growth factor treatment induced HDAC7 degradation via PLCgamma-IP3K (phospholipase Cgamma-inositol-1,4,5-trisphosphate kinase) signal pathway and partially rescued HDAC7-mediated suppression of proliferation. Moreover, vascular endothelial growth factor stimulation suppressed the binding of HDAC7 with beta-catenin, disrupting the complex and releasing beta-catenin to translocate into the nucleus.

CONCLUSIONS: These findings demonstrate that HDAC7 interacts with beta-catenin keeping ECs in a low proliferation stage and provides a novel insight into the mechanism of HDAC7-mediated signal pathways leading to endothelial growth.

4. MMP28 gene expression is regulated by Sp1 transcription factor acetylation.

Biochem J. 2010 Apr 14;427(3):391-400.

Swingler TE, Kevorkian L, Culley KL, Illman SA, Young DA, Parker AE, Lohi J, Clark IM.

MMP-28 (epilysin) is a recently cloned member of the MMP (matrix metalloproteinase) family. It is highly expressed in the skin by keratinocytes, the developing and regenerating nervous system and a number of other normal human tissues, as well as a number of carcinomas. The MMP28 promoter has previously been cloned and characterized identifying a conserved GT-box that binds Sp1/Sp3 (specificity proteins 1 and 3) proteins and is essential for the basal expression of the gene. The present study demonstrates that MMP28 expression is induced by HDAC (histone deacetylase) inhibitors and that this effect is mediated through the GT-box. Transient transfection assays have shown that the induction of MMP28 expression by the HDAC inhibitior TSA (trichostatin A) is mediated via Sp1 at the GT-box. Immunoprecipitation experiments have shown that the acetylation of Sp1 and Sp3 is increased by TSA treatment; however, no effect on DNA binding was observed. Histone acetyltransferases such as p300 and P/CAF [p300/CREB (cAMP-response-element-binding protein)-binding protein-associated factor] increased induction of the MMP28 promoter by Sp1. Knockdown of HDAC1 using siRNA (small interfering RNA) also induces the MMP28 promoter. Oligonucleotide pulldown identified STRAP (serine/threonine kinase receptor-associated protein) as a further protein recruited to the MMP28 promoter and acting functionally with Sp1.

CRIPTO

1. Nodal promotes growth and invasion in human gliomas.

Oncogene. 2010 Apr 12. [Epub ahead of print]. 12 April 2010; doi:10.1038/onc.2010.55. PMID: 20383200 [PubMed - as supplied by publisher]

Lee CC, Jan HJ, Lai JH, Ma HI, Hueng DY, Gladys Lee YC, Cheng YY, Liu LW, Wei HW, Lee HM.

Uncontrolled growth and diffused invasion are major causes of mortality in patients with malignant gliomas. Nodal has been shown to have a central role in the tumorigenic signaling pathways of malignant melanoma. In this study, we show that grade IV human glioma cell lines expressed different levels of Nodal, paralleled to the potential for cell invasiveness. Treatment of glioma cell lines with recombinant Nodal (rNodal) increased matrix metalloproteinase 2 (MMP-2) secretion and cell invasiveness. The ectopic expression of Nodal in GBM glioma cells that expressed Nodal at low level resulted in increased MMP-2 secretion, enhanced cell invasiveness, raised cell proliferation rates in vitro, increased tumor growth in vivo, and was associated with poor survival in a mice xenograft model. In contrast, the knockdown of Nodal expression in U87MG glioma cells with high Nodal expression level had reduced MMP-2 secretion, less cell invasiveness, lower tumor growth in vivo and longer lifespan in mice with U87MG/shNodal cell xenografts. In addition, Nodal knockdown promoted the reversion of malignant glioma cells toward a differentiated astrocytic phenotype. Furthermore, our data support the notion that Nodal may regulate glioma progression through the induction of the leukemia inhibitory factor (LIF) and Cripto-1 through activated Smad.Oncogene advance online publication,

MYOSTATIN INHIBITORS

None during the period.

BIOMATERIAL DRUG DELIVERY

1. Shape-memory polymers as a technology platform for biomedical applications.

Expert Rev Med Devices. 2010 May;7(3):357-79.

Lendlein A, Behl M, Hiebl B, Wischke C.

Polymeric materials are clinically required for medical devices, as well as controlled drug delivery systems. Depending on the application, the polymer has to provide suitable functionalities, for example, mechanical functions or the capability to actively move, so that an implant can be inserted in a compact shape through key-hole incisions and unfold to its functional shape in the body. Shape-memory polymers, as described herein regarding their general principle, compositions and architectures, have developed to a technology platform that allows the tailored design of such multifunctionality. In this way, defined movements of implants triggered either directly or indirectly, tailored mechanical properties, capability for sterilization, biodegradability, biocompatibility and controlled drug release can be realized. This comprehensive review of the scientific and patent literature illustrates that this technology enables the development of novel medical devices that will be clinically evaluated in the near future.

2. Biomaterial-based technologies for brain anti-cancer therapeutics and imaging.

Biochim Biophys Acta. 2010 Apr 18. [Epub ahead of print]

Orive G, Ali OA, Anitua E, Pedraz JL, Emerich DF.

Treating malignant brain tumors represents one of the most formidable challenges in oncology. Contemporary treatment of brain tumors has been hampered by limited drug delivery across the blood-brain barrier (BBB) to the tumor bed. Biomaterials are playing an increasingly important role in developing more effective brain tumor treatments. In particular, polymer (nano)particles can provide prolonged drug delivery directly to the tumor following direct intracerebral injection, by making them physiochemically able to cross the BBB to the tumor, or by functionalizing the material surface with peptides and ligands allowing the drug-loaded material to be systemically administered but still specifically target the tumor endothelium or tumor cells themselves. Biomaterials can also serve as targeted delivery devices for novel therapies including gene therapy, photodynamic therapy, anti-angiogenic and thermotherapy. Nanoparticles also have the potential to play key roles in the diagnosis and imaging of brain tumors by revolutionizing both preoperative and intraoperative brain tumor detection, allowing early detection of pre-cancerous cells, and providing real-time, longitudinal, non-invasive monitoring/imaging of the effects of treatment. Additional efforts are focused on developing biomaterial systems that are uniquely capable of delivering tumor-associated antigens, immunotherapeutic agents or programming immune cells in situ to identify and facilitate immune-mediated tumor cell killing. The continued translation of current research into clinical practice will rely on solving challenges relating to the pharmacology of nanoparticles but it is envisioned that novel biomaterials will ultimately allow clinicians to target tumors and introduce multiple, pharmaceutically relevant entities for simultaneous targeting, imaging, and therapy in a unique and unprecedented manner.

3. Mechanism of Protein Release from Polyelectrolyte Multilayer Microcapsules.

Biomacromolecules. 2010 Apr 20. [Epub ahead of print]

She Z, Antipina MN, Li J, Sukhorukov GB.

The development of polyelectrolyte multilayer microcapsules as a delivery system containing bioactive compounds strongly depends on understanding of the major factors that influence capsules' loading and release of incorporated substances. Mechanism of protein release from biocompatible polyelectrolyte multilayer microcapsules has been examined using two different approaches of protein encapsulation: (i) "preloading" via coprecipitation of tetramethylrhodamine isothiocyanate (TRITC)-labeled bovine serum albumin (BSA) (TRITC-BSA) into CaCO(3) particles followed by multilayer assembly and (ii) "postloading" of TRITC-BSA in preformed empty capsules templated on pure CaCO(3) particles taken in the same amount as in "preloading" approach. Polysaccharides (alginate (Alg) or dextran sulfate (Dex)) and polyarginine (PAr) were used as layer constituents. On the basis of the effects of capsule shell composition and thickness, method of protein encapsulation, volume

of the surrounding medium, and frequency of medium refreshment on protein release profile, we reveal a mechanism of protein release. The key phenomenon determining the protein release is the property of multilayer polyelectrolyte shells relating to the entrapping and accumulation of protein molecules. The results obtained together with the suggested mechanism of capsule loading and protein release allow us to propose the use of polyelectrolyte microcapsules as a depot system to supply and maintain a defined level of macromolecular drug concentration in surrounding medium.

4. Nanostructured hyaluronic acid-based materials for active delivery to cancer.

Expert Opin Drug Deliv. 2010 Apr 5. [Epub ahead of print]

Ossipov DA.

Importance of the field: Active targeting of bioactive molecules by physicochemical association with hyaluronic acid (HA) is an attractive approach in current nanomedicine because HA is biocompatible, non-toxic and non-inflammatory. Areas covered in this review: This review focuses on synthesis, physicochemical characterization and biological properties of different nanoparticulate delivery systems that include HA in their structures. Chemically based approaches to the delivery of small molecule drugs, proteins and nucleic acids in which they become chemically or physically bound to hyaluronic acid are reviewed, including the use of molecular HA conjugates and nanocarriers. The systems are considered in terms of intracellular delivery to different cultured cells that express HA-specific receptors (hyaladherines) differently. The in vivo biodistribution and therapeutic effect of these systems are discussed. What the reader will gain: Different synthetic methodologies for preparations of HA-based nanoparticles are presented extensively. HA nanoparticulate systems of various structures can be compared with respect to their in vitro assays and in vivo biodistribution. Take home message: To make HA useful as an intravenous targeting carrier, strategies have to be devised to: reduce HA clearance from the blood; suppress the HA uptake by liver and spleen; and provide tumor-triggered mechanisms of release of an active drug from the HA carrier.

5. The effects of strontium-substituted bioactive glasses on osteoblasts and osteoclasts in vitro.

Biomaterials. 2010 May;31(14):3949-56. Epub 2010 Feb 18.

Gentleman E, Fredholm YC, Jell G, Lotfibakhshaiesh N, O'Donnell MD, Hill RG, Stevens MM.

Bioactive glasses (BG) which contain strontium have the potential to combine the known bone regenerative properties of BG with the anabolic and anti-catabolic effects of strontium cations. Here we created a BG series (SiO(2)-P(2)O(5)-Na(2)O-CaO) in which 0-100% of the calcium was substituted by strontium and tested their effects on osteoblasts and osteoclasts in vitro. We show that ions released from strontium-substituted BG enhance metabolic activity in osteoblasts. They also inhibit osteoclast activity by both reducing tartrate resistant acid phosphatase activity and inhibiting resorption of calcium phosphate films in a dose-dependent manner. Additionally, osteoblasts cultured in contact with BG show increased proliferation and alkaline phosphatase activity with increasing strontium substitution, while osteoclasts adopt typical resorption morphologies. These results suggest that similarly to the osteoporosis drug strontium ranelate, strontium-substituted BG may promote an anabolic effect on osteoblasts and an anti-catabolic effect on osteoclasts. These effects, when combined with the advantages of BG such as controlled ion release and delivery versatility, may make strontium-substituted BG an effective biomaterial choice for a range of bone regeneration therapies.

6. Antibiotic-releasing porous polymethylmethacrylate constructs for osseous space maintenance and infection control.

Biomaterials. 2010 May;31(14):4146-56. Epub 2010 Feb 13.

Shi M, Kretlow JD, Nguyen A, Young S, Scott Baggett L, Wong ME, Kasper FK, Mikos AG.

The use of a strategy involving space maintenance as the initial step of a two-stage regenerative medicine approach toward reconstructing significant bony or composite tissue defects in the craniofacial area, preserves the void volume of bony defects and could promote soft tissue healing prior to the subsequent definitive repair.

One of the complications with a biomaterial-based space maintenance approach is local infection, which requires early, effective eradication, ideally through local antibiotic delivery. The purpose of this study is to develop a dual function implant material for maintaining osseous space and releasing an antibiotic to eliminate local infection in bony defects. Colistin, a polymyxin antibiotic, was chosen specifically to address infections with Acinetobacter species, the most common pathogen associated with combat-related traumatic craniofacial injuries. Porous polymethylmethacrylate (PMMA) constructs incorporating poly(lactic-co-glycolic acid) (PLGA) microspheres were fabricated by mixing a clinically used bone cement formulation of PMMA powder and methylmethacrylate liquid with a carboxymethylcellulose (CMC) hydrogel (40 or 50 wt%) to impart porosity and PLGA microspheres (10 or 15 wt%) loaded with colistin to control drug release. The PMMA/CMC/PLGA construct featured mild setting temperature, controllable surface/bulk porosity by incorporation of the CMC hydrogel, reasonably strong compressive properties, and continuous drug release over a period of 5 weeks with total drug release of 68.1-88.3%, depending on the weight percentage of CMC and PLGA incorporation. The concentration of released colistin was well above its reported minimum inhibitory concentration against susceptible species for 5 weeks. This study provides information on the composition parameters that enable viable porosity characteristics/drug release kinetics of the PMMA/CMC/PLGA construct for the initial space maintenance as part of a two-stage regenerative medicine approach.

7. Structure-property relationships of silk-modified mesoporous bioglass scaffolds.

Biomaterials. 2010 May;31(13):3429-38. Epub 2010 Feb 1.

Wu C, Zhang Y, Zhu Y, Friis T, Xiao Y.

Porous mesopore-bioglass (MBG) scaffolds have been proposed as a new class of bone regeneration materials due to their apatite-formation and drug-delivery properties; however, the material's inherent brittleness and high degradation and surface instability are major disadvantages, which compromise its mechanical strength and cytocompatibility as a biological scaffold. Silk, on the other hand, is a native biomaterial and is well characterized with respect to biocompatibility and tensile strength. In this study we set out to investigate what effects blending silk with MBG had on the physiochemical, drug-delivery and biological properties of MBG scaffolds with a view to bone tissue engineering applications. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were the methods used to analyze the inner microstructure, pore size and morphology, and composition of MBG scaffolds, before and after addition of silk. The effect of silk modification on the mechanical property of MBG scaffolds was determined by testing the compressive strength of the scaffolds and also compressive strength after degradation over time. The drug-delivery potential was evaluated by the release of dexamethasone (DEX) from the scaffolds. Finally, the cytocompatibility of silk-modified scaffolds was investigated by the attachment, morphology, proliferation, differentiation and bone-relative gene expression of bone marrow stromal cells (BMSCs). The results showed that silk modification improved the uniformity and continuity of pore network of MBG scaffolds, and maintained high porosity (94%) and large-pore size (200-400 microm). There was a significant improvement in mechanical strength, mechanical stability, and control of burst release of DEX in silk-modified MBG scaffolds. Silk modification also appeared to provide a better environment for BMSC attachment, spreading, proliferation, and osteogenic differentiation on MBG scaffolds.

8. Repair of large osteochondral defects in rabbits using porous hydroxyapatite/collagen (HAp/Col) and fibroblast growth factor-2 (FGF-2).

J Orthop Res. 2010 May;28(5):677-86.

Maehara H, Sotome S, Yoshii T, Torigoe I, Kawasaki Y, Sugata Y, Yuasa M, Hirano M, Mochizuki N, Kikuchi M, Shinomiya K, Okawa A.

Articular cartilage has a limited capacity for self-renewal. This article reports the development of a porous hydroxyapatite/collagen (HAp/Col) scaffold as a bone void filler and a vehicle for drug administration. The scaffold consists of HAp nanocrystals and type I atelocollagen. The purpose of this study was to investigate the efficacy of porous HAp/Col impregnated with FGF-2 to repair large osteochondral defects in a rabbit model. Ninety-six cylindrical osteochondral defects 5 mm in diameter and 5 mm in depth were created in the femoral trochlear groove of the right knee. Animals were assigned to one of four treatment groups: porous HAp/Col impregnated with 50 microl of FGF-2 at a concentration of 10 or 100 microg/ml (FGF10 or FGF100 group); porous HAp/Col with 50 microl of PBS (HAp/Col group); and no implantation (defect group). The defect areas

were examined grossly and histologically. Subchondral bone regeneration was quantified 3, 6, 12, and 24 weeks after surgery. Abundant bone formation was observed in the HAp/Col implanted groups as compared to the defect group. The FGF10 group displayed not only the most abundant bone regeneration but also the most satisfactory cartilage regeneration, with cartilage presenting a hyaline-like appearance. These findings suggest that porous HAp/Col with FGF-2 augments the cartilage repair process.

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Number of Words: 45 078 (approx.)
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