

Expert Review of Hematology

EDITORIAL

Individualizing treatment for Waldenstrom's macroglobulinemia

DRUG PROFILES

Clinical activity of laromustine (Onorigin™) in hematologic malignancies

Bosutinib: a dual SRC/ABL kinase inhibitor for chronic myeloid leukemia

Prophylaxis of venous thrombosis in congenital antithrombin deficiency

MEETING REPORT

2nd International Congress on Leukemia, Lymphoma and Myeloma

KEY PAPER EVALUATION

Recombinant erythropoiesis-stimulating agents and cancer mortality

REVIEWS

Adoptive T-cell therapy for B-cell malignancies

Biology and treatment of follicular lymphoma

Prognostic tools in follicular lymphomas

Prognostic molecular markers in myelodysplastic syndromes

Noninvasive approach for the management of fetus hemolytic disease

Late complications after hematopoietic stem cell transplantation

EXPERT
REVIEWS

PROPERTY OF THE
NATIONAL
LIBRARY OF
MEDICINE



Expert Review of

Hematology

October 2009

Expert Rev. Hematol. 2(5)**Editorial**

- 473 Individualizing treatment for Waldenstrom's macroglobulinemia
J Stedman, A Roccarorene & IM Ghobrial

News in brief

- Safety concerns halt trial of sildenafil in sickle cell
- Acute myeloid leukemia genome sequenced
- Helixate® approved for hemophilia A children
- Transfusion confusion still abounds
- Double award glory for leukemia pioneer

Drug Profiles

- 481 Clinical activity of larmustine (Onrigin™) in hematologic malignancies
Y Alvarado, R Swords, KR Kelly & FJ Giles
- 489 Bosutinib: a dual SRC/ABL kinase inhibitor for the treatment of chronic myeloid leukemia
G Keller, P Schafhausen & TH Brummendorf
- 499 Antithrombin- α for the prophylaxis of venous thrombosis in congenital antithrombin deficiency
A Fyfe & RC Tait

Meeting Report

- 509 2nd International Congress on Leukemia, Lymphoma and Myeloma
M Arat, GH Özsan, MN Yenerel, M Cetin & M Özcan

Key Paper Evaluation

- 513 Two faces for Janus: recombinant human erythropoiesis-stimulating agents and cancer mortality
R Addeo, M Caraglia, N Frega & SD Prete

Reviews

- 517 Adoptive T-cell therapy for B-cell malignancies
M Hudecek, LD Anderson Jr, T Nishida & SR Riddell
- 533 Biology and treatment of follicular lymphoma
PP Piccaluga, MR Sapienza, C Agostinelli, C Sagrarnoso, C Mannu, E Sabattini, PL Zinzani & SA Pileri
- 549 Prognostic tools in follicular lymphoma
S Luminari, MC Cox, A Montanini & M Federico
- 563 Prognostic molecular markers in myelodysplastic syndromes
J Neukirchen, R Haas & U Germing
- 577 Noninvasive approach for the management of hemolytic disease of the fetus
S Illanes & P Soothill
- 583 Late complications after hematopoietic stem cell transplantation
A Tichelli, A Rovó, J Passweg, CP Schwarze, MTV Lint, M Arat & G Socié

Appendices

- 602 Glossary
- 604 Indices
- 609 Acknowledgements
- 610 Author Guidelines
- Calendar

EXPERT REVIEWS

Managing Director

James Drake

Publisher

David Hughes

Editorial Director

Elisa Manzotti

COMMISSIONING

DEPARTMENT

Head of Commissioning

Victoria Lane

Commissioning Editor

Andy Palmer

Drug Profiles Editor

Tarek Kattan

PRODUCTION DEPARTMENT

Head of Production

Karen Rowland

Managing

Production Editor

Philip Chapman

Production Editor

Hannah Morton

Assistant

Production Editors

Eleanor Samarasekera

Maria Nicholas

Expert Review of Hematology

Editorial Board

The Editorial Board is drawn from the leading forces in research and academia

Aledort LM, Mt Sinai School of Med., NY, USA

Aplan P, National Cancer Institute, MD, USA

Ataga K, Univ. of North Carolina, NC, USA

Byrd JC, The Ohio State Univ., OH, USA

Cappellini MD, Univ. of Milan, Italy

Castaman G, San Bortolo Hospital, Italy

Cortes J, MD Anderson Cancer Ctr., TX, USA

Dimopoulos MA, Univ. of Athens, Greece

Dreyling M, Univ. of Munich, Germany

Engert A, Univ. Hosp. of Cologne, Germany

Giles FJ, Cancer Ther. & Res. Center, TX, USA

Grever M, The Ohio State Univ., OH, USA

Harousseau J-L, Ctr. Hosp. Univ. Hôtel-Dieu, France

Hochhaus A, Univ. of Heidelberg, Germany

Lambertenghi Delilieri G, Univ. of Milan, Italy

Leen AM, Baylor College of Med., TX, USA

Machin SM, Univ. College London, UK

Murphy M, National Blood Service, UK

O'Connor O, Columbia Univ. Med. Ctr, NY, USA

Orlowski RZ, MD Anderson Cancer Ctr., TX, USA

Palmblad J, Karolinska Institutet, Sweden

Pileri S, Bologna Univ. School of Med., Italy

Prentice A, Royal Free Hospital, UK

Richardson P, Dana Farber Cancer Inst., MA, USA

Roboz GJ, Weill Medical College NY, USA

Schiffer C, Barbara Ann Karmanos Cancer Inst., USA

Stone RM, Dana Farber Cancer Inst., MA, USA

Valent P, Med. Univ. of Vienna, Austria

Disclaimer: Whilst every effort is made by the Publisher and Editorial Board to ensure that no inaccurate or misleading data, opinions or statements appear in this journal, they wish to make it clear that the data and opinions appearing herein are the responsibility of the contributor concerned. Accordingly, the Publisher, Editorial Board and their respective employees, officers and agents accept no liability whatsoever for the consequences of any inaccurate or misleading data, opinions or statements.

Copyright: Conditions of sale: *Expert Review of Hematology* may be circulated only to those members of staff who are employed at the site at which the subscription is taken out. Readers are reminded that, under internationally agreed copyright legislation, photocopying of copyright materials is prohibited other than on a limited basis for personal use. Thus making copies of any article published in *Expert Review of Hematology* is a breach of the law and can be prosecuted.

ENQUIRIES

Editorial

Elisa Manzotti, Editorial Director
e.manzotti@expert-reviews.com

Subscriptions

Simon Boisseau, Sales Director
s.boisseau@expert-reviews.com

Marketing

Craig Canham, Marketing Manager
c.canham@expert-reviews.com

Reprint

Sam Cavana, Reprint Sales Manager
s.cavana@expert-reviews.com

Permissions

Leela Ripton, Permissions Coordinator
l.ripton@expert-reviews.com

Online

Online Access Team
subscriptions@expert-reviews.com

Indexing: Chemical Abstracts

www.expert-reviews.com

part of the
Miltényi Ex. 1011 Page 3

fsg

“...provides commentary and analysis to elucidate best clinical practice in hematology...”

Advanced molecular research techniques have transformed hematology in recent years. With improved understanding of hematologic diseases, we now have the opportunity to research and evaluate new biological therapies, drugs and drug combinations, treatment schedules and novel approaches, including stem cell transplantation. Further advances in our knowledge regarding the formation and function of blood cells and blood-forming tissues should ensue, and it will be a major challenge for hematologists to adopt these new paradigms and develop integrated strategies to define the best possible patient care.

Expert Review of Hematology provides commentary and analysis to elucidate best clinical practice in hematology and to translate advances in research – in areas such as immunology, stem cell research, and cell and gene therapy – into the clinical context.

Coverage includes:

- Hematological malignancies
- Anemias
- Thrombosis and hemostasis
- Bone marrow and hematopoietic stem cell transplantation
- Immunodeficiency syndromes
- Autoimmune diseases
- Marrow failure syndromes
- Neutropenia
- Platelet disorders
- Transfusion medicine

Subscription Options

Institutional subscriptions

Expert Review of Hematology is available in print, electronic or print and electronic formats, and pricing will depend on your organization type (academic, corporate, hospital, etc). Please contact info@expert-reviews.com for more details.

Global e-access licenses are available on request and attract considerable discounts from standard site license fees. For further details on global access licenses, please contact Simon Boisseau: s.boisseau@expert-reviews.com

Consortia pricing

Expert Reviews welcomes discussion with all consortia, and offers flexible packages and discounted prices. If you have specific questions or would like a quote please contact info@expert-reviews.com for more details.

Personal subscriptions

Personal subscriptions are currently available to all Expert Reviews journals. Payment must be made from a personal credit card registered to a home address. Print subscriptions will only be sent to a personal address. Please contact info@expert-reviews.com for our personal order form, or order online at www.future-science-group.com/subscriptions

Ordering Information

Please contact your local sales representative to place an order:

Worldwide

Expert Reviews Ltd
Unitec House, 2 Albert Place, London
N3 1QB, UK
T: +44 (0)20 8371 6080
F: +44 (0)20 8343 2313
E: subscriptions@expert-reviews.com

North America

E: sales.us@expert-reviews.com

Latin America and the Caribbean

Systems Link International
T: +1 305 826 6191
E: informacion@systemsint.info

China

Charlesworth China
T: +86 106 779 1601
E: sales@charlesworth.com.cn

Japan

Technomics Inc.
T: +81 (03) 3666 2952
E: sales@technomics.co.jp

Asia (excluding Japan & China)

iGroup Asia Pacific
T: +852 2572 7228
E: info.kh@igroup.com

Subscription Rates	Print & Online			Online Only		
	£ GBP	€ Euro	US\$	£ GBP	€ Euro	US\$
Journal (6 issues)						
Academic & Hospital	605	755	1160	535	670	1030
Corporate, Government & NGO Centers	Please contact info@expert-reviews.com for more details, or contact your local sales representative					

Reprints

Article reprints are available through our reprint service.
Please contact Sam Cavana: s.cavana@expert-reviews.com

Expert Reviews titles endorse the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, issued by the International Committee for Medical Journal Editors, and Code of Conduct for Editors of Biomedical Journals, produced by the Committee on Publication Ethics.

This information is also available at www.expert-reviews.com

Manuscript submission & processing

Expert Reviews titles publish a range of article types, including solicited and unsolicited reviews, perspectives and original research articles. Receipt of all manuscripts will be acknowledged within 1 week and authors will be notified as to whether the article is to progress to external review. Initial screening of articles by internal editorial staff will assess the topicality and importance of the subject, the clarity of presentation, and relevance to the audience of the journal in question. If you are interested in submitting an article, or have any queries regarding article submission, please contact the Managing Commissioning Editor for the journal (contact information can be found on our website at: www.expert-reviews.com). For new article proposals, the Managing Commissioning Editor will require a brief article outline and working title in the first instance. We also have an active commissioning program whereby the Commissioning Editor, under the advice of the Editorial Advisory Panel, solicits articles directly for publication.

External peer review

Through a rigorous peer review process, Expert Reviews titles aim to ensure that reviews are unbiased, scientifically accurate and clinically relevant. All articles are peer reviewed by three or more members of the International Advisory Board or other specialists selected on the basis of experience and expertise. Review is performed on a double-blind basis – the identities of peer reviewers and authors are kept confidential. Peer reviewers must disclose potential conflicts of interests that may affect their ability to provide an unbiased appraisal (see Conflict of Interest Policy below). Peer reviewers complete a referee report form, provide general comments to the editor and both general and specific comments to the author(s).

Where an author believes that an editor has made an error in declining a paper, they may submit an appeal. The appeal letter should clearly state the reasons why the author(s) considers the decision to be incorrect and provide detailed, specific responses to any comments relating to the rejection of the review. Further advice from members of the journal's Editorial Advisory Panel external experts will be sought regarding eligibility for re-review.

Revision

Most manuscripts require some degree of revision prior to acceptance. Authors should provide two copies of the revised manuscript – one of which should be highlighted to show where changes have been made. Detailed responses to reviewers' comments, in a covering letter/email, are also required. Review manuscripts may be accepted at this point or may be subject to further peer review. The final decision on acceptability for publication lies with the journal editor.

Post-acceptance

Accepted review manuscripts are edited by the in-house Expert Reviews editorial team. Authors will receive proofs of their article for approval and sign off and will be asked to sign a transfer of copyright agreement, except in circumstances where the author is ineligible to do so (e.g. government employees in some countries).

Author disclosure & conflict of interest policy

Authors must state explicitly whether potential conflicts do or do not exist (e.g. personal or financial relationships that could influence their actions) and any such potential conflict of interest (including sources of funding) should be summarized in a separate section of the published review. Authors must disclose whether they have received writing assistance and identify the sources of funding for such assistance. Authors declaring no conflict of interest are required to publish a statement to that effect within the article.

Authors must certify that all affiliations with or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in their manuscript have been disclosed. Please note that examples of financial involvement include: employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending and royalties. This list is not exclusive of other forms of financial involvement. Details of relevant conflicts of interests (or the lack of) must be declared in the 'Disclosure' section of the manuscript for all listed authors.

External peer reviewers must disclose any conflicts of interest that could bias their opinions of the manuscript, and they should disqualify themselves from reviewing specific manuscripts if they believe it appropriate. Should any such conflict of interest be declared, the journal editor will judge whether the reviewer's comments should be recognized or will interpret the reviewer's comments in the context of any such declaration.

Ethical conduct of research

For studies involving data relating to human or animal experimental investigations, appropriate institutional review board approval is required and should be described within the article. For those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki should be followed. For investigations involving human subjects, authors should explain how informed consent was obtained from the participants involved.

Patients' rights to privacy

Patients have a right to privacy that should not be infringed without informed consent. Identifying information should not be included unless the information is essential for scientific purposes and the patient (or parent or legal guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. When informed consent has been obtained it should be indicated in the manuscript.

In attempting to maintain patient anonymity, identifying details should be omitted where they are not essential. However, patient data should never be amended or falsified. Informed consent should be obtained whenever there is any doubt that anonymity can be assured.

Use of personal communications & unpublished data

Where an individual is identified within a review as a source of information in a personal communication or as a source for unpublished data, authors should include a signed statement of permission from the individual(s) concerned and specify the date of communication.

Clinical trial registration

Expert Reviews titles prefer to publish clinical trials that have been included in a clinical trials registry that is accessible to the public at no charge, is electronically searchable, is open to prospective registrants and is managed by a not-for-profit organization, such as www.clinicaltrials.gov (sponsored by the United States National Library of Medicine). Whilst referees will take registration status into account, all well designed and presented trials and corresponding data will be considered for publication.

Errata/corrigenda

Mistakes by either editor or author should be identified wherever possible and an erratum or corrigendum published at the earliest opportunity. We will attempt to contact the author of the original article to confirm any error, and publish an appropriate erratum or corrigendum at the earliest opportunity.

Permissions for reproduced or adapted material

Authors must acknowledge the origin of all text, figures, tables or other information that has been adapted or reproduced from other publications. Authors must provide a copy of the original source documents and should submit permission from the authors of the original work and the original publishers for unlimited use in all markets and media (that includes both electronic and print use in any language).

Duplicate publication/submission & plagiarism

All manuscripts submitted to Expert Reviews titles are considered for publication on the understanding that they have not been published previously elsewhere or are under consideration for publication elsewhere. The journal may, however, consider republication of a paper previously published in a language other than English, subject to prominent disclosure of the original source and with any necessary permission. Authors will be asked to certify that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under their authorship has been published or is being considered for publication elsewhere, except as described in an attachment, and copies of closely related manuscripts are provided. The use of published or unpublished ideas, words or other intellectual property derived from other sources without attribution or permission, and representation of such as those of the author(s) is regarded as scientific misconduct and will be addressed as such.

Misconduct

If misconduct by authors or reviewers is suspected, either pre- or post-publication, action will be taken. An explanation will be sought from the party or parties considered to be involved. If the response is unsatisfactory, then an appropriate authority will be asked to investigate fully. Expert Reviews will make all reasonable attempts to obtain a resolution in any such eventuality and correct the record or archive as necessary.

EXPERT
REVIEWSAdoptive T-cell therapy for
B-cell malignancies*Expert Rev. Hematol.* 2(5), 517–532 (2009)**Michael Hudecek,
Larry D Anderson Jr,
Tetsuya Nishida and
Stanley R Riddell***

*Author for correspondence

Fred Hutchinson Cancer
Research Center, 1100 Fairview
Avenue N, D3-100, Seattle,
WA 98109, USA

Tel.: +1 206 667 5249

Fax: +1 206 667 7983

sriddell@fhcrc.org

The success of allogeneic hematopoietic cell transplantation (HCT) for B-cell malignancies is evidence that these tumors can be eliminated by T lymphocytes. This has encouraged the development of specific adoptive T-cell therapy, both for augmenting the anti-tumor effect of HCT and for patients not undergoing HCT. T cells that are capable of recognizing antigens expressed on malignant B cells may be recruited from the endogenous repertoire or engineered to express tumor-targeting receptors. Critical insights into the qualities of T cells that enable their persistence and function *in vivo* have been derived, and obstacles to effective T-cell-mediated tumor eradication are being elucidated. These advances provide the tools to translate adoptive T-cell transfer into reliable clinical therapies.

KEYWORDS: adoptive T-cell therapy • allogeneic hematopoietic cell transplantation • central memory T cell • chimeric antigen receptor • immunoglobulin idiotype • graft-versus-host disease • graft-versus-tumor effect • minor histocompatibility antigen • T-cell receptor • tumor antigen

The potential to use immune-based therapies for human malignancies is attractive because of the specificity of antibody and T-cell recognition. The most profound advances have been made in the development of antibodies as therapeutics, and several monoclonal antibodies that recognize molecules on the surface of cancer cells are being used in human cancer therapy. Antibodies that target CD20 and CD52 are now routinely employed in standard therapeutic regimens for subsets of patients with B-cell malignancies [1–3]. The development of effective T-cell therapy for human malignancy either through vaccination or by adoptive T-cell transfer, which refers to the isolation, expansion and reinfusion of tumor-reactive T cells, has been substantially more challenging. The difficulties in developing T-cell-based immunotherapies are due, in part, to the inability of current vaccines to reproducibly elicit effective tumor-reactive T-cell responses, and the complexity of deriving and expanding tumor-reactive T cells *ex vivo* that have the capacity to persist and function following adoptive transfer. Despite these obstacles, the exquisite ability of T cells to distinguish diseased from normal cells has encouraged the continued investigation of strategies to employ T cells as therapeutic agents.

There is evidence from allogeneic hematopoietic cell transplantation (HCT) that advanced B-cell malignancies are susceptible to a T-cell-mediated graft-versus-tumor (GVT) effect,

although GVT activity cannot yet be reproducibly separated from graft-versus-host disease (GVHD) [4–7]. The demonstration that B-cell tumors are recognized by T cells has provided optimism that donor T cells specific for tumor-associated antigens might be isolated, expanded and administered to the patient to augment the GVT effect; or that autologous T cells might be elicited or engineered to recognize tumor-associated antigens, without the need for allogeneic HCT. Engineering of tumor reactive T cells can be accomplished by gene transfer techniques that introduce a T-cell receptor (TCR) with specificity for peptide fragments of intracellular proteins displayed on class I and class II MHC molecules expressed by the tumor, or a chimeric antigen receptor (CAR) that consists of a single-chain antibody fragment (scFv) specific for a B-cell surface molecule linked to the ζ -chain of the CD3/TCR complex [8,9]. This review will discuss the rationale and theoretical framework for developing adoptive T-cell therapy for B-cell malignancies, the obstacles that have been encountered, and the directions that are currently being taken for clinical translation.

**GVT effect of allogeneic HCT in
B-cell malignancies**

Allogeneic HCT provides a potentially curative therapy for a variety of hematologic malignancies, including many B-cell tumors. Originally,

allogeneic HCT was developed as a method of rescuing patients from the lethal toxicity of high doses of myeloablative chemoradiotherapy administered to achieve greater tumor-cell killing than could be achieved with conventional doses of chemotherapy [10], and was used as a treatment of last resort for patients with refractory leukemia, including B-lineage acute lymphoblastic leukemia (B-ALL). Consistent with the prediction from murine models, that immune recognition of tumor cells could contribute to tumor eradication, human allogeneic HCT for B-ALL was accompanied by a T-cell mediated GVT effect [11–13]. A GVT effect of allogeneic HCT has subsequently been confirmed in other B-cell malignancies, including chronic lymphocytic leukemia (B-CLL), multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) [14–18]. The myeloablative chemotherapy regimen contributes substantially to tumor control but it is now realized that the curative potential of this procedure is a result of the immunologic elimination of malignant cells. This is evident from the strikingly lower relapse rate and increased leukemia-free survival rate in patients receiving an allogeneic HCT compared with syngeneic HCT [12,13]. There are several factors that limit the success of allogeneic HCT and efforts to improve outcome are focused on reducing toxicity due to conditioning and GVHD, and augmenting the GVT effect (TABLE 1).

Donor lymphocyte infusions for B-cell malignancies

A critical role for donor T cells in the GVT effect was demonstrated by studies by Kolb *et al.* who investigated the use of donor lymphocyte infusions (DLIs) in patients with leukemia relapse after allogeneic HCT [19]. Durable complete remissions were achieved in 10–40% of patients with B-ALL, B-CLL, MM and lymphomas [20–22]. This compares with response rates of up to 70% in patients with relapsed chronic myeloid leukemia (CML) and it has been hypothesized that the superior ability of CML cells to differentiate into antigen presenting dendritic cells (DCs) and prime anti-tumor T-cell responses may be responsible for these differences in outcome [19,23]. To improve the outcome of patients with B-cell malignancies receiving DLIs, strategies such as the administration of pre-DLI chemotherapy or the use of *ex vivo*-activated donor lymphocytes are being explored. The induction

of GVHD remains a major complication associated with the use of donor lymphocytes. Although some patients receiving DLI achieve complete remissions in the absence of clinically evident GVHD, most responding patients develop acute and chronic GVHD suggesting the antigens that are recognized on tumor cells are often shared with other tissues. The risk of GVHD can be attenuated in a subset of patients by using gradually escalating doses of DLI but this strategy is most effectively applied in slowly progressive malignancies [24–26].

Reduced-intensity conditioning regimens for allogeneic HCT

Allogeneic HCT employing myeloablative conditioning is restricted to younger and medically fit patients to avoid excessive mortality from toxicities related to chemoradiotherapy. The median age at diagnosis for B-CLL and MM is over 60 years and these patients often have comorbidities due to their age, underlying malignancy or prior chemotherapy. This limitation to the use of allogeneic HCT was overcome by the development of reduced-intensity conditioning (RIC) regimens that use low doses of chemoradiotherapy to immunosuppress the recipient sufficiently to prevent rejection of the donor stem and T-cell graft, and enable a GVT effect to mediate tumor eradication [27,28]. RIC-HCT has been effective in several indolent B-cell malignancies, including B-CLL, MM and lymphoma, although aggressive lymphoma and B-ALL are less responsive [5–7,29]. Similar to the observations made in patients receiving myeloablative HCT, the anti-tumor efficacy of RIC-HCT is highly correlated with GVHD, which often requires long-term immunosuppressive therapy and is a major cause of morbidity and mortality. Therefore, the development of approaches that could be incorporated with HCT, such as the adoptive transfer of T cells that can specifically target antigens that are preferentially or selectively expressed on the tumor to augment the GVT effect without inducing GVHD, is a high priority of current research efforts.

Minor histocompatibility antigens as targets for the GVT effect

In the context of an allogeneic HCT from an HLA-identical donor, T-cell recognition of minor histocompatibility antigens (minor H antigens) is responsible for GVHD and has been implicated in the GVT effect. Minor H antigens result from genetic polymorphisms between the HCT recipient and the corresponding HLA-identical stem cell donor [30]. The most common mechanism for generating a minor H antigen is a non-synonymous single nucleotide polymorphism (SNP) that results in a peptide presented by HLA-molecules on recipient cells to which the HLA-identical donor is not tolerant and can be recognized as 'non-self' by donor CD8⁺ and CD4⁺ T cells [31]. Other mechanisms for generating minor H antigens including alternative splicing of peptide fragments, and differential protein expression as a consequence of gene deletion have also been identified [32,33].

Reduced-intensity conditioning-HCT relies almost exclusively on immune elimination of tumor cells and provides an opportunity to dissect the specificity of tumor-reactive T cells that develop

Table 1. Factors that limit the success of allogeneic hematopoietic cell transplantation.

Obstacle	Strategy
Toxicity due to the conditioning regimen	Employ reduced-intensity conditioning regimens
Graft-versus-host disease	Improve drug regimens for immunosuppression Targeted suppression of alloreactive T-cell activation or function Remove alloreactive T cells from the donor stem cell graft
Tumor relapse	Adoptive transfer of tumor-reactive T cells Vaccination to elicit tumor-reactive T-cell responses <i>in vivo</i>

after HCT, and identify minor H or tumor-associated antigens that are the targets of the GVT response. In a study at the Fred Hutchinson Cancer Research Center (FHCRC) we analyzed the temporal kinetics and specificity of T cells that develop after non-myeloablative-HCT for B-CLL because large numbers of tumor cells could be stored pre-transplant and used to assess recognition by donor T cells that developed in the recipient post-transplant [34]. We found that CD8⁺ and CD4⁺ T-cell responses directed against minor H antigens expressed by B-CLL developed in all patients that achieved sustained tumor regression and correlated with the clearance of tumor cells from peripheral blood, bone marrow and lymph nodes. By contrast, patients with progressive B-CLL after HCT failed to develop T cells that recognized leukemic cells, even though they often developed GVHD, suggesting the failure to eradicate the tumor in these patients was because alloreactive T cells only recognized antigens that were not shared by target tissues of GVHD and the tumor (FIGURE 1) [34]. The kinetics with which tumor-reactive T cells developed in responding patients after RIC-HCT varied from several weeks to 1 year, however once established, these T cells persisted long-term, indicating that immunologic memory was established. Analysis of the specificity of CD8⁺ T cells that recognized B-CLL in patients that achieved a complete remission demonstrated that multiple minor H antigens were being targeted, including those that were broadly expressed on both leukemic cells and nonhematopoietic tissues [34].

A focus of current work in the field of allogeneic HCT is the identification of minor H antigens that are limited in their expression to normal and malignant hematopoietic cells, and are absent on GVHD target tissues. Several minor H antigens that meet this prerequisite have been discovered, including HA-1, ACC-1 and LRH-1 [31,35,36], which are selectively expressed on all hematopoietic cells; and PANE-1, HB-1 and CD19, which are exclusively expressed on B-lineage cells and could be used to target B-cell malignancies [37–39]. However, the utility of an individual hematopoietic-restricted minor H antigen as a target for T-cell therapy is determined by the frequency of the HLA allele that presents the antigen and the requirement that the patient and donor be mismatched for the minor H antigen, in the appropriate direction. For HA-1, the most extensively studied minor H antigen, the disparity rate is estimated to be 4.2–8.5% in HLA-A2⁺ sibling pairs and 6.8–12.2% in HLA-A2 matched unrelated pairs [40]. These data illustrate a problem for the translation of minor H antigen specific T-cell therapy into clinical practice

with the panel of minor H antigens that are presently known, since only a few patients could actually benefit from this strategy. This obstacle will only be resolved by the discovery of additional minor H antigens. The HapMap project has identified over 10⁴ SNPs in the human genome that confer amino acid sequence changes in coding regions and this database has enabled the use of genome association studies for minor H antigen discovery, which should rapidly expand the number of minor H antigens at our disposal [41]. A second obstacle for targeting minor H antigens is the need to derive and expand minor H antigen-specific T cells from the donor for adoptive transfer in every case. A potential solution discussed later in this review is the isolation of TCR genes from high-avidity minor H antigen-specific T cells, construction of gene transfer vectors and engineering of primary donor T cells for transfer in suitable patients.

Adoptive immunotherapy with T cells specific for nonpolymorphic tumor-associated antigens

Ideally, nonpolymorphic antigens expressed on B-cell tumors could be targeted using autologous T cells to circumvent the need to find a suitable donor for allogeneic HCT and the complications associated with HCT. In our analysis of T-cell responses that developed after

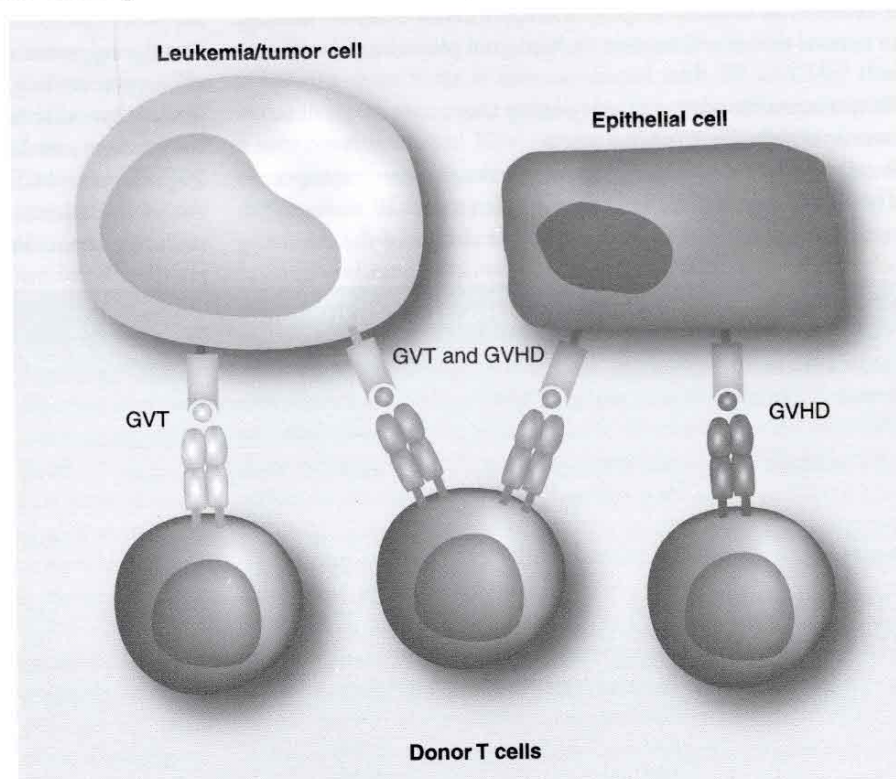


Figure 1. Expression of minor H antigens on tumor cells and epithelium dictate GVHD and GVT activity mediated by donor T cells. Minor H antigens that are selectively expressed on tumor cells but not on epithelium can serve as targets for a selective GVT response in the absence of GVHD. T cells specific for minor H antigens expressed on both tumor and epithelium can mediate both a GVT effect and GVHD, those specific for minor H antigens on tumor but not epithelium mediate a selective GVT response, while those specific for minor H antigens on epithelium but not on tumor only mediate GVHD.
GVHD: Graft-versus-host disease; GVT: Graft-versus-tumor.

allogeneic RIC-HCT for B-CLL, we were surprised to find that a proportion of T-cell clones were specific for nonshared and shared determinants that are only expressed on the recipient B-CLL, and not on Epstein–Barr virus (EBV)-transformed B cells [34]. Additional studies are in progress to characterize these putative tumor-specific antigens that are targeted as part of the anti-B-CLL response after transplant, and to determine whether they represent tumor-specific mutations or nonpolymorphic proteins. Several candidate tumor-specific proteins have already been identified in distinct subtypes of B-cell malignancies and are being pursued as targets for T-cell therapy. These include the immunoglobulin idiotype (Id), the bcr–abl protein in Philadelphia chromosome-positive B-ALL [42], and EBV-associated proteins that are expressed in EBV-positive Hodgkin disease (HD), Burkitt's lymphoma (BL), and EBV-induced lymphoproliferations that develop in severely immunocompromised patients. In addition to tumor-specific determinants that are restricted in their expression to the B-cell tumor, many proteins have been found to be overexpressed or aberrantly expressed in malignant B cells and to be present at much lower levels in normal tissues (TABLE 2). Tolerance to many of these tumor-associated self-proteins is not complete and it has been possible to isolate T cells that selectively lyse tumor cells. Whether such T cells can be used for adoptive therapy without excessive damage to normal tissues will require evaluation in pilot clinical trials.

Tumor-specific antigens: targeting the immunoglobulin idiotype

B-cell malignancies derive from a single B-cell clone that expresses a unique immunoglobulin (Ig) molecule on the cell surface. The variable regions of the heavy and light chains of the tumor Ig

contain determinants that are specific for the malignant clone and are referred to as Id. The Id is a specific target for immunotherapy and passive immunotherapy with custom-made monoclonal anti-Id antibodies inducing an anti-tumor effect in up to 66% of patients, and long-lasting tumor regressions achieved in some cases [43]. However, this approach failed to completely eradicate all malignant B cells, and some patients relapsed owing to outgrowth of a mutated tumor Id-variant that had lost the epitope recognized by the monoclonal antibody [43,44]. T-cell recognition of Id-derived epitopes has also been demonstrated, and Id-specific T cells were shown to eradicate B-cell tumors in murine myeloma models [45].

Based on the partial efficacy of passive antibody therapy in humans and animal model data demonstrating anti-tumor activity of Id-specific T cells, it was logical to evaluate vaccination regimens that might elicit both antibody and T-cell responses. Clinical trials have focused on patients with follicular lymphoma (FL), which is sufficiently indolent to provide time to produce the vaccine and to develop an immune response after vaccination. Studies by Kwak *et al.* showed that immunization with autologous Id protein generated Id-specific humoral and cellular immune responses in patients with FL [46]. In subsequent trials, adjuvant cytokines and Id-loaded DCs were incorporated into vaccine regimens in an effort to improve immunogenicity and efficacy [47,48]. In a Phase I/II study by Bendandi *et al.*, in which granulocyte macrophage colony-stimulating factor was added to the vaccine, anti-Id antibody responses were induced in 15 out of 20 patients and CD8⁺/CD4⁺ T-cell responses were induced in 19 out of 20 patients. A subset of these patients achieved sustained molecular remissions of their lymphoma, providing evidence for

an anti-tumor effect of vaccination [48]. A subsequent Phase II study in patients who had relapsed after initial chemotherapy, confirmed the immunogenicity of this approach and suggested a clinical benefit of vaccination based on a prolonged duration of complete response to reinduction chemotherapy [49]. Both humoral and cellular immune responses may be important in anti-tumor effects following anti-Id vaccination, although a fraction of patients who achieved complete remission after Id vaccination did so in the absence of detectable antibody responses [48]. A recent retrospective analysis of FL patients who had received Id vaccinations addressed the question whether the superior clinical outcome associated with an anti-Id immune response also resulted in an improved overall survival (OS). The study showed that the generation of an antibody response correlated with an improved OS at 10 years, whereas the generation of an anti-Id T-cell response was surprisingly not associated with improved OS [50]. This could reflect

Table 2. Nonpolymorphic antigens in B-cell malignancies.

Antigen class	Example	Tumor expression	Ref.
Immunoglobulin idiotype	CDR region of Id	Individual Id expressed in each B-cell malignancy	[51]
Viral antigens	EBV latent proteins (e.g., EBNA-1, 3, LMP-1,2)	EBV-LPD, Hodgkin disease	[65,68]
Chromosome translocation	Bcr/abl	Philadelphia chromosome-positive B-ALL	[42]
Overexpressed proteins	Bcl-2	B-CLL	[149]
	Fibromodulin	B-CLL	[74]
	Mdm2	B-CLL	[73]
	RHAMM/CD168	B-CLL	[150]
	Survivin	B-CLL	[75]
	DKK1	MM	[78]
	HM1.24	MM	[77]
	PRAME	MM, B-CLL	[150]
	SPAN-XB	MM	[76]
Aberrantly expressed proteins	WT-1	MM, B-ALL	[80,81]
	Cancer-testes antigens (e.g., NY-ESO-1, LAGE, MAGE)	MM	[80]

B-ALL: B-cell acute lymphoblastic leukemia; B-CLL: B-cell chronic lymphocytic leukemia; CDR: Complementarity determining region; EBV: Epstein–Barr virus; Id: Idiotype; LPD: Lymphoproliferative disease; MM: Multiple myeloma.

the sensitivity of assays to detect Id-reactive T cells compared with those to evaluate humoral immunity. Characterization of anti-Id T-cell reactivity has mapped the immunogenic epitopes within the hypervariable complementarity determining regions of the Ig heavy chain [51]. Of interest, *in vitro* studies have also suggested that Ig framework-derived peptides can function as cytotoxic T-cell epitopes [52], although mapping of the specificity of responses in vaccinated patients has yet to reveal recognition of framework regions.

The potential benefit of Id vaccination in FL has now been investigated in three Phase III studies [53]. The results of these trials have not yet been published but preliminary information that has been released indicates that only one trial showed a significant benefit in disease-free survival for vaccinated patients [53]. This study included 177 previously untreated patients with FL who achieved a complete remission after induction chemotherapy. The median disease-free survival for patients who received Id vaccination was 33.8 months versus 21.1 months in the control arm. This advantage in disease-free survival has not been observed in the two other studies [53]. The publication of data from these trials is awaited with interest, and may provide insight into what factors contribute to the differences in outcome following Id vaccination.

Compared with the success of Id vaccination in FL, the induction of anti-Id immunity in MM and aggressive lymphoma has been more challenging, and clinical responses have only been observed in a minority of patients [54,55]. The neutralization of anti-Id-antibodies by circulating myeloma protein and immune-escape mechanisms, such as the secretion of TGF- β and IL-10, by myeloma cells may compromise the priming and/or function of anti-Id T-cell responses [56]. Additionally, studies in murine myeloma models showed that Id-specific T cells are progressively deleted from the T-cell repertoire with increasing serum levels of myeloma protein [56,57]. Even if reactive T cells can be elicited, the functional phenotype may be critical in anti-tumor activity, with cytotoxic T lymphocytes and Th1 cells inhibiting tumor growth and Th2 cells promoting tumor progression [58].

Idiotype-specific T cells can be generated *in vitro* through stimulation with peptide-pulsed or Id-transduced autologous DCs, as well as through genetic introduction of chimeric anti-Id receptors, but efforts to employ adoptive T-cell transfer to target Ids have been limited to animal model studies [59,60]. The requirement to produce a customized Id product from each patient, to use as a reagent for antigen presentation, combined with the complexity of reproducibly deriving a T-cell product for adoptive therapy from each patient makes such an effort challenging. The use of vaccination with Id-protein or Id-loaded DC to elicit Id-specific T cells that could then be more readily isolated and expanded *ex vivo* may provide a solution to the latter problem, and facilitate studies to determine if vaccination combined with adoptive T-cell transfer could improve upon the results achieved with vaccination alone [55]. Furthermore, vaccination with tumor lysate-loaded DCs may represent a strategy to induce or reactivate a T-cell response with reactivity against multiple tumor antigens, including the Ig-Id [61].

Targeting EBV-associated B-cell malignancies with T-cell therapy

The lymphotropic EBV was first discovered in cultured lymphocytes from BL [62], and has also been detected in HD. The oncogenic potential of EBV is evident in severely immunocompromised HCT and solid-organ transplant patients who develop EBV-driven B-cell proliferations, termed EBV-associated lymphoproliferative disease. These tumors develop as a consequence of inadequate T-cell surveillance, and express the full range of latent cycle EBV antigens, including Epstein-Barr nuclear antigen (EBNA)-3A, -3B and -3C proteins, which serve as targets for T cells in immunocompetent donors. Indeed, EBV-associated lymphoproliferative disease that occurred in recipients of T-cell depleted allogeneic HCT can be successfully treated by the adoptive transfer of unselected lymphocytes from the respective EBV-seropositive donor, although this approach is complicated by GVHD [63]. To avoid GVHD, Rooney *et al.* isolated EBV-specific T cells from the peripheral blood of HCT donors by repeated *in vitro* stimulation and demonstrated that adoptive transfer of only the EBV-reactive T cells promoted regression of established EBV-driven lymphomas, and prevented the development of tumors when administered prophylactically [64]. The introduction of a marker gene into a subset of transferred EBV-specific T cells was used to track infused T cells and demonstrated that the cells persisted long-term *in vivo* [64].

Epstein-Barr virus is also associated with BL and HD that occur in immunocompetent individuals, but these tumors express a very restricted set of EBV antigens, that presumably facilitates their escape from host immunity. EBNA-1 is the only EBV protein expressed in BL but is not recognized efficiently by CD8⁺ T cells [65,66]. HD also expresses EBNA-1, and the weakly immunogenic latent membrane proteins (LMP-1) and -2. Although LMP-1 and -2 are weakly immunogenic, strategies for isolating and expanding autologous T cells specific for these antigens from patients with HD have been developed [67,68]. Pilot studies in which T cells targeting these EBV antigens have been expanded and adoptively transferred to patients with HD have shown that the EBV-reactive T cells migrate to tumor sites and result in tumor regression in a subset of patients [69].

Targeting nonmutated tumor-associated antigens derived from self-proteins

Microarray studies that have compared the gene-expression profile of malignant B cells to their respective normal counterparts have uncovered highly expressed genes in the various histologic types of B-cell tumors [70–72]. Algorithms that predict peptide binding to MHC have identified potentially immunogenic peptide epitopes that might serve as targets for T-cell therapy in several overexpressed proteins, including MDM2, fibromodulin and survivin in B-CLL [73–75]; NY-ESO-1, SPAN-XB, DKK1, HM1.24 and WT-1 in MM [76–80] and WT-1 in B-ALL [81]. Tumor-reactive CD8⁺ T cells that recognize these and other self-peptides have been isolated from normal donors using *in vitro* culture techniques [73–78,82,83]. However, it is often difficult to isolate T cells with high affinity to these self-antigens from patients who have been pretreated with cytotoxic chemotherapy that affects T-lymphocyte

numbers and function. Additionally, it remains to be determined whether targeting antigens that are also expressed in some normal tissues with T-cell therapy may lead to undesired toxicity.

Efforts to target self-proteins expressed on B-cell tumors by adoptive therapy have initially focused on WT-1 and NY-ESO-1, which are only expressed at low levels in very few normal tissues but are highly expressed in tumor cells of some patients. WT-1 is expressed at high levels in myeloid leukemias and B-ALL and at very low levels on CD34⁺ hematopoietic stem cells. WT-1-specific T-cell responses have been elicited in normal donors and implicated in the GVT effect against B-ALL after allogeneic HCT [83,84]. A WT-1 peptide vaccine increased the frequency of WT-1-tetramer-positive T cells and induced an anti-tumor response in a subset of leukemia patients without adverse events [85]. Techniques for the isolation of T cells specific for multiple epitopes in WT-1 from normal donors have been described [82,83], and clinical trials of adoptive T-cell therapy targeting WT-1 have been initiated.

NY-ESO-1 is a member of the cancer testis (CT) family of proteins that are normally only present in the testis and the placenta, but are upregulated in a variety of tumor types, including a subset of patients with MM and melanoma. NY-ESO-1 was found to be the most immunogenic CT antigen and both spontaneous and vaccine-induced immune responses against NY-ESO-1 have been reported in cancer patients [86,87]. A clinical trial of adoptive T-cell therapy for melanoma with autologous CD4⁺ NY-ESO-1-specific T-cell clones resulted in a remission in one

out of nine patients, illustrating the potential for T cells of this specificity to mediate anti-tumor activity [88]. These results have implications for the development of adoptive T-cell therapy for MM, since many members of the CT antigen family, including NY-ESO-1, are expressed in myeloma cells and these proteins have been implicated in tumor progression [89].

Genetic modification: endowing T cells with receptors that target tumor antigens

Although polymorphic and tumor-associated antigens have been identified for B-cell malignancies and mediate anti-tumor activity *in vitro* or in preclinical models, their translation into clinical trials is hampered in many cases by the failure to reproducibly isolate high-affinity T cells from patients or healthy donors that are specific for these antigens. The promise of adoptive T-cell therapy will only be realized if methods for the reproducible production of tumor-reactive T cells for therapy are developed. The genetic modification of T cells provides a potential solution for this problem, since T cells can be engineered to express a TCR capable of recognizing the desired antigen with high affinity or to express an artificial CAR that is specific for a tumor cell surface molecule (FIGURE 2).

T-cell receptor gene transfer

The specificity of T-cell recognition is provided by the TCR, and the transfer of *TCR-α* and *-β* genes into recipient T cells can endow the cells with the antigen specificity of the introduced TCR [90]. Thus, one approach to derive T cells for therapy of B-cell tumors is to introduce TCR genes from clones with specificity for a viral, minor H antigen, or nonpolymorphic tumor antigen into T lymphocytes of a patient [8,91–94]. The *TCR-α* and *-β* genes can be derived from the rare T cells that have been isolated with high affinity for the desired antigen, or from transgenic mice that express human HLA alleles and have been immunized with the human protein to generate a high-affinity murine T-cell response from which the murine HLA-restricted TCRs can be cloned [93].

Gene transfer into T cells

The majority of TCR transfer studies have employed γ -retroviral vectors (RV) based on murine leukemia virus or murine sarcoma virus, or self-inactivating lentiviral vectors (LVs) [95,96]. A characteristic of RV is that cell division is required for vector integration following infection [97]. By contrast, LVs have the ability to transduce a variety of slowly or even nondividing cells, including unstimulated T cells in the absence of TCR activation [98]. For the

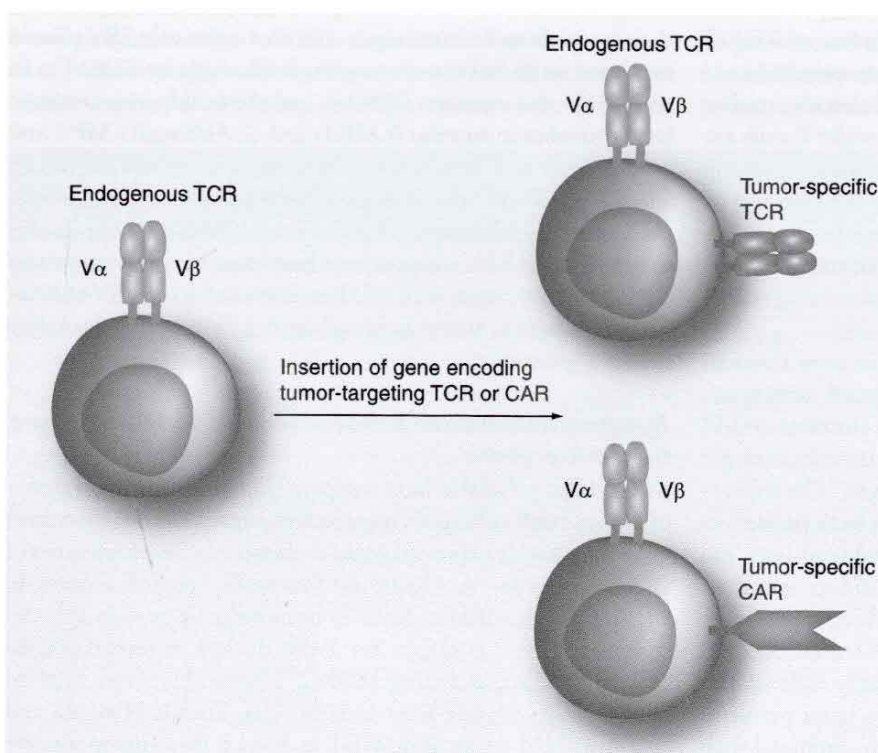


Figure 2. Gene transfer can retarget primary T cells to recognize tumor cells. Schematic demonstration of the engineering of bispecific T cells by inserting the α - and β -genes of a TCR specific for a tumor-associated antigen, or a gene encoding a CAR constructed of a single-chain antibody fragment fused to TCR signaling domains. CAR: Chimeric antigen receptor; TCR: T-cell receptor.

genetic modification of T cells, this may be an advantage since TCR activation and sustained proliferation required for RV transduction may impair the ability of transduced T cells to persist after adoptive transfer and reduce their anti-tumor activity. Both RV and LV preferentially integrate into transcriptionally active genes, and entail a risk of insertional mutagenesis and transformation of modified T cells, although LVs are considered to be less genotoxic [99,100]. The development of lymphoproliferative complications due to dysregulated proto-oncogene expression in a gene therapy trial that used engineered hematopoietic stem cells in patients with X-linked severe combined immunodeficiency (SCID), highlighted the risk related to cell transformation as a consequence of gene insertion and has led to the development of self-inactivating vectors with reduced mutagenic potential (Box 1) [101–103]. Compared with hematopoietic stem cells, mature T cells have been found to be more resistant to oncogene transformation and so far, no case of malignant transformation has been reported in adoptive immunotherapy trials that employed T cells genetically modified with suicide genes or gene markers [104]. Nonviral gene-delivery systems, such as electroporation into T cells have also been developed, however, the expression that can be achieved with plasmid DNA may vary considerably among T cells and is only transient after electroporation of RNA [105,106]. A potentially attractive nonviral system for stable gene transfer is the sleeping beauty (SB) transposon technology. SB is a synthetic transposable element that has been generated from the ancestral Tc1/mariner-like transposon in fish [107]. DNA transposons encode a transposase flanked by inverted terminal repeats that contain transposase binding sites. Any gene of interest flanked by the inverted terminal repeats can undergo transposition into the host genome via a cut and paste mechanism. SB transposase has been shown to enable stable gene transfer into primary human T cells and its utilization for TCR gene transfer is anticipated [108].

The only experience using TCR gene transfer to generate T cells for adoptive therapy of human cancer has been in metastatic melanoma. In the first reported study, two out of 15 patients experienced objective tumor regressions after receiving autologous T lymphocytes engineered to express a TCR specific for the melanocyte differentiation antigen MART-1 [92]. Although this study established the therapeutic potential of genetically modified T cells for the treatment of human malignancies, issues were identified that need to be addressed to improve the results. These include safe methods for gene delivery that achieve adequate and stable TCR transgene expression, strategies for minimizing pairing of the introduced TCR chains with the endogenous TCR

chains that might result in a deleterious specificity, and introducing the TCR into T cells that can establish a durable functional T-cell response *in vivo* after adoptive transfer.

Achieving adequate TCR expression, pairing & avidity

T-cell receptor transgene cassettes that allow the coupled transcription of both genes under control of the same promoter yielding a single coding mRNA molecule have been developed. These vectors utilize an internal ribosomal entry site or virus-derived sequences that encode self-cleaving peptides, such as 2A to promote coordinate translation of the mRNA into two separate α - and β -chain proteins. Recent studies that compared both features favored 2A elements, which allowed translation of the TCR- α and β chains at an almost equimolar ratio and provided optimal expression of the TCR [96].

A significant concern with TCR transfer is the potential for cross-pairing of transferred with endogenous TCR chains, which could result in the formation of hybrid receptors, with potentially autoreactive specificity. Preferential pairing of the two introduced TCR- α and β chains can be achieved by replacing the human constant regions of the TCR- α and β chains with murine constant regions, or by incorporating cysteine residues in positions that promote disulfide bonds between the introduced chains [109]. As an alternative method, the introduction of TCR- α and β chains into $\gamma\delta$ T cells has been proposed to prevent TCR mispairing. However, the low frequency of $\gamma\delta$ T cells in the peripheral blood and their preferential homing to the mucosa of the GI tract make them less attractive for adoptive immunotherapy of B-cell malignancies [110].

T-cell receptor chains specific for self-proteins are often of low affinity, and it may be necessary to improve affinity for therapeutic efficacy. This can be accomplished for a TCR of known specificity through directed *in vitro* evolution, enabling the engineering of TCRs with the ability to bind HLA-bound peptides at picomolar concentrations [111], or by high-throughput screening of TCR that have been mutated randomly to identify higher affinity pairs.

Chimeric antigen receptors

In 1997, the monoclonal anti-CD20-antibody rituximab was approved as the first therapeutic antibody for cancer treatment and has since revolutionized the treatment of CD20-positive B-cell tumors. The combination of rituximab with standard chemotherapy has significantly improved response and survival rates compared with chemotherapy alone [112,113]. The potential

Box 1. Potential genotoxicity of gene transfer into somatic cells.

Gene transfer into somatic cells can trigger oncogenesis as a consequence of the upregulation of cellular proto-oncogenes. Insertional mutagenesis has resulted in the development of leukemia in a subset of patients with X-linked severe combined immunodeficiency who were treated with hematopoietic stem cells that had been corrected by retrovirus-mediated gene transfer. Mature T cells have been shown to be relatively resistant to oncogene transformation as a result of retroviral insertion [104], and vector design may further reduce the genotoxic risk from vector integration and improve the safety of gene-modified T-cell therapy of malignancy. Nevertheless, this issue remains a concern for the clinical translation of therapeutics with gene-modified T cells. An additional approach to improve safety is to concurrently introduce a mechanism for conditional cell suicide, either using a drug that activates a death program or a monoclonal antibody to invoke killing by antibody-dependent cellular cytotoxicity [142–145].

to combine the specificity of monoclonal antibodies with the advantages of a durable cellular immune response spurred the development of CARs that could be expressed in T cells. CARs typically consist of a scFv that incorporates the heavy and light variable chains (VH and VL, respectively) of a monoclonal antibody that recognizes a tumor cell molecule fused to the ζ -chain of the CD3/TCR complex to trigger T-cell activation and cytotoxicity. To improve activation after engagement of the CAR, a costimulatory signal can be provided by the addition of CD28 or other costimulatory signaling domains to the ζ -chain [114,115]. An important advantage of CAR-modified T cells is their ability to recognize tumor cells without the requirements of MHC restriction, removing obstacles, such as defects in antigen processing and low levels of MHC expression on malignant cells, that might limit the efficacy of conventional or TCR-modified T cells.

Several B-cell lineage surface molecules are retained on B-cell tumors and represent attractive targets for CAR-modified T-cell therapy. The feasibility of adoptive immunotherapy with autologous CD20-specific CAR-modified T cells has been studied in patients with NHL and mantle cell lymphoma. In a trial of seven patients, two maintained a previous complete response, one achieved a partial response and four had stable disease [116]. Modified T cells persisted for up to 5–9 weeks after infusion in some patients but at a very low level. Improving the magnitude and duration of the response achieved by adoptive transfer will be important to improve efficacy, particularly for the treatment of patients with a larger tumor burden. Other surface molecules that are expressed on B-cell tumors have been targeted with CAR-modified T cells in preclinical studies, including CD19 and CD22 [117,118], and clinical trials with CD19-redirectioned T cells are now in progress in several laboratories for B-ALL, B-CLL and lymphoma.

Clinical trials of T-cell therapy using CAR-modified T cells should provide insight into potential limitations of this approach. One concern is that transferred T cells might be eliminated prematurely due to a host immune response to the murine VH and VL fragments of CARs that are derived from murine antibodies. Another issue identified in preclinical work is that the stoichiometry of antibody binding to tumor cells is different from that of the TCR/MHC interaction and might not provide for optimal T-cell activation or survival *in vivo*. Clinical trials are required to determine how significant these issues will be. Many molecules being targeted on B-cell malignancies by CAR-modified T cells are also expressed on normal B cells, and successful therapy is likely to result in a B-cell deficiency, unless strategies are developed for the conditional elimination of transferred T cells after tumor eradication is complete.

Qualitative properties of T cells required for effective adoptive therapy

The efficacy of adoptive T-cell therapy requires that the transferred tumor-reactive T cells home to sites of tumor, mediate effector functions in the tumor environment, and persist sufficiently long *in vivo* to eradicate the majority or even all of the malignant cells. Preclinical models and clinical trials have shown

that modifying the environment into which T cells are transferred and selecting T cells with distinct properties for adoptive transfer are important for achieving optimal anti-tumor effects.

Homing of T cells to tumor sites

The ability of adoptively transferred T cells to migrate to and penetrate large tumor masses has been shown to be an impediment to effective therapy of solid tumors in murine models [119], and may reflect alterations in homing receptors expressed on T cells, dysregulated expression of leukocyte adhesion molecules on the tumor vasculature and biophysical properties of the tumor environment that may limit T-cell infiltration [120,121]. There are limited data on the migration of human T cells administered to patients with malignancy and B-cell tumors in particular. Adoptively transferred T cells specific for EBV antigens expressed on EBV-associated post-transplant lymphoproliferative disease or HD were readily identified in tumor biopsies, suggesting that homing may be less of an obstacle for tumors that originate in lymph nodes or bone marrow, particularly if high levels of transferred T cells can be achieved and sustained in the blood [69]. Many tumors produce chemokines to attract and educate cells to have immunosuppressive functions, thus, in situations where homing of effector T cells is limited, it may be possible to improve their migration to tumor sites by introducing chemokine receptors that respond to factors produced in the tumor environment [122].

Local suppression of effector function in the tumor environment

It is increasingly apparent that the tumor environment is hostile to the development and function of effector T cells as a consequence of the local recruitment of Tregs and myeloid suppressor cells, tumor cell expression of ligands for inhibitory receptors, and/or secretion of molecules that inhibit effector T-cell function. The precise immune evasion strategies employed by B-cell malignancies are beginning to be elucidated and will be instructive for designing modifications to adoptively transferred T cells or the host to overcome immune evasion. For example, PD-L1 is present in HD and MM and promotes anergy and apoptosis of T cells after binding to programmed death-1 (PD-1) [123,124]. PD-1 expression was elevated in tumor-infiltrating and peripheral T cells of HD patients and blockade of the PD-1 signaling pathway restored IFN- γ production of tumor-infiltrating T cells, suggesting that such an approach might improve the efficacy of adoptively transferred tumor-reactive T cells [124]. Other immune escape strategies have been identified in HD and MM, including the production of TGF- β and IL-10, and strategies to render T cells resistant to these negative regulators have been described [56,125]. Finally, loss of tumor antigen expression and downregulation of HLA and costimulatory molecules, occur in B-cell malignancies, suggesting that targeting multiple epitopes might be necessary to prevent the emergence of antigen loss variants under immune selective pressure provided by adoptive T-cell transfer [126].

Recent studies by Gribben *et al.* have identified direct effects on gene-expression profiles of CD4⁺ and CD8⁺ T cells induced by cell-to-cell contact with B-CLL cells [127]. The affected genes

are involved in cytoskeleton formation, vesicle trafficking and cytotoxicity, and the altered gene expression in T cells is accompanied by defective synapse formation with target cells, leading to impaired recognition of malignant B-CLL cells. The formation of a functional immune synapse between T cells and B-CLL could be partially restored *in vitro* with the immunomodulatory drug lenalidomide, suggesting its potential use to promote recognition of tumor cells by adoptively transferred T cells [128,129].

Improving persistence of adoptively transferred tumor-reactive T cells with cytokines

Studies in animal models of adoptive T-cell therapy for leukemia have demonstrated that transferred T cells must persist for long periods to mediate complete tumor rejection. Efforts to translate T-cell therapy to human malignancies have required the *in vitro* isolation and expansion of T cells, which may render them less fit to survive and function *in vivo*. One of the first strategies that improved T-cell survival after adoptive transfer was the administration of IL-2 [130]. Administration of both low-dose and high-dose IL-2 transiently improved the persistence of transferred T cells, however high-dose IL-2 was accompanied by significant side effects [131].

IL-7 and IL-15 are used less often than IL-2 for expanding T cells *in vitro* but have been shown to be critical for survival and proliferation of memory T cells *in vivo* [132,133]. IL-7 and IL-15 levels become elevated in lymphopenia and induce the proliferation of T cells to restore homeostasis [134]. It was therefore hypothesized that the induction of lymphopenia might lead to prolonged persistence of adoptively transferred T cells by reducing the competition for homeostatic cytokines, minimizing the influence of Tregs and other suppressors, and creating space in the lymphoid compartment of the recipient for the transferred T cells. The use of lymphodepleting chemotherapy alone or with irradiation prior to the transfer of melanoma-specific T cells resulted in better persistence and expansion of transferred T cells *in vivo*, as well as improved tumor infiltration and anti-tumor activity [135]. Further clinical studies will help to elucidate whether the administration of homeostatic cytokines alone may have a similar effect on transferred T cells and circumvent the toxicity associated with lymphodepleting chemoradiotherapy.

Selecting T cells with the intrinsic capacity to persist & function after adoptive transfer

Clinical trials of adoptive T-cell therapy for human viral diseases after allogeneic HCT using T-cell clones or polyclonal T cells derived from a healthy donor with prior exposure to the pathogen demonstrated that transferred T cells could persist long-term

in vivo [64,136]. This proved not to be the case when autologous tumor-reactive T cells were isolated from cancer patients and used to treat human malignancies, and poor persistence of transferred T cells probably contributed to the lack of sustained anti-tumor efficacy [116,130,135]. An explanation for these apparently discrepant results is now emerging from work in animal models, which suggest that the distinct transcriptional programs of naive and subsets of memory T cells impart qualitative differences that are retained in effector progeny and influence cell fate after adoptive transfer (Box 2).

Discrete phenotypic and functional subsets of T cells have been identified, including naive (TN), effector (TE), central memory (TCM) and effector memory (TEM) T cells [137]. After TCR engagement by antigen *in vivo*, TN cells undergo proliferation and programmed differentiation, resulting in the generation of large numbers of TE cells, most of which die as antigen is cleared, leaving a small pool of functionally distinct TCM and TEM cells that persist long-term and respond to antigen re-exposure by differentiating into a new wave of TE cells [137,138]. It has generally been assumed that TE cells isolated from TN, TCM or TEM subsets would behave similarly and have equivalent efficiency when used for adoptive T-cell therapy. Recent data in a nonhuman primate model in which antigen-specific TE cells derived from either TCM or TEM were reinfused into animals has cast doubt on this assumption. In this study, T-cell clones derived from TCM, but not those derived from TEM cells were capable of persisting long term, reacquiring the phenotype of memory cells and populating memory T-cell niches *in vivo* after adoptive transfer [139]. These results demonstrate that some of the progeny of clonally derived TE cells retain intrinsic programming of the parental cell of origin and provide an explanation for the inconsistent persistence of transferred T cells when unselected T cells are used as the source to generate tumor-specific T cells for adoptive immunotherapy. Moreover, these findings suggest that selecting T cells from the TCM pool for the introduction of T-cell or chimeric antigen receptors that target B-cell malignancies may ensure more uniform persistence after cell transfer. An alternative approach that has been employed is to use T cells specific for a virus, such as EBV or CMV for genetic modification, since many of these cells will be derived from TCM. This strategy also enables signaling through the endogenous TCR to potentially amplify and maintain the transferred T cells *in vivo* and has been used in preclinical studies with EBV-specific T cells expressing an anti-CD30 ζ CAR to target HD and with some success in clinical trials with EBV-specific T cells expressing a CAR specific for neuroblastoma [140,141].

Box 2. Distinct programming of T cells used for adoptive therapy.

It has been suggested previously that at least some memory T cells have properties of self-renewal and differentiation, attributes typically assigned to stem cells [146]. A recent study in mice demonstrated that the initial division of a naive T cell after antigen encounter is asymmetric and endows each of the daughter cells with distinct properties that dictate their ability to function as memory cells [147]. A putative memory T stem cell has also been described in a murine model of graft-versus-host disease [148]. Taken together, this work illustrates that even similar T-cell subsets exhibit distinct programming, and has focused greater attention on how properties of T cells other than tumor specificity might influence their efficacy in adoptive immunotherapy.

Expert commentary

The application of adoptive T-cell therapy for human malignancies, including B-cell tumors has long held attraction due to the potential for specific elimination of tumor cells without the toxicities associated with chemotherapy and radiation. There is convincing evidence from allogeneic HCT that B-cell malignancies are susceptible to elimination by T cells, however the translation of this knowledge into specific adoptive T-cell therapy has been impeded by the lack of readily available antigens to target in B-cell tumors, with the exception of Ig-Id or EBV antigens in the subset of malignancies that express EBV proteins. Efforts to identify polymorphic and nonpolymorphic tumor antigens expressed on B-cell malignancies that can be recognized by T cells have led to studies of T-cell therapy in preclinical models, and clinical trials of this approach have been initiated. The engineering of T cells to express a TCR or CAR that targets antigens and surface molecules that are broadly expressed by B-cell malignancies can overcome the obstacle of isolating tumor-reactive T cells from the endogenous repertoire, and has facilitated the initiation of clinical trials by several groups in both aggressive and indolent B-cell tumors. Critical insight has been derived into qualitative properties of T cells that ensure persistence after adoptive transfer. The evaluation of data from well-designed clinical trials and ongoing studies in animal models will determine the potential toxicity, efficacy and limitations of adoptive T-cell therapy for B-cell malignancies, and will direct subsequent studies that may lead to the establishment of adoptive T-cell transfer as a useful and broadly applicable modality.

Five-year view

The field of adoptive T-cell therapy is currently undergoing a significant resurgence, due in part to the proven efficacy of this approach in melanoma, the opportunities provided by molecular

profiling of tumors to identify target antigens, and the feasibility of isolating or engineering T cells of defined specificity. Allogeneic HCT will remain a mainstay of therapy for B-cell malignancies owing to its proven curative potential, and efforts in several laboratories to identify polymorphic antigens expressed on B-cell tumors using genome-association studies will identify minor H antigens that can be targeted selectively with T-cell therapy to augment GVT reactivity without causing GVHD. If this endeavor is successful, the use of less toxic RIC regimens might be extended to younger patients with more aggressive B-cell tumors. Clinical trials of adoptive T-cell therapy for B-cell malignancies in the nontransplant setting, including those that use TCR- or CAR-modified T cells, have already been initiated and will be completed in the next 5 years. These trials will provide data on the safety and efficacy of this approach that will be used to design subsequent trials, and determine whether it is appropriate and how best to incorporate T-cell therapy into conventional therapeutic regimens. In patients who fail to respond to T-cell therapy, it will be important to analyze whether the failure of therapy reflects intrinsic properties of effector T cells, or systemic or local suppression of function in the tumor-bearing patient to provide insight into the subset of patients that can benefit most by T-cell therapy, and to derive strategies for improving outcome in patients less likely to respond.

Financial & competing interests disclosure

This work was supported by NIH grants CA18029 (Stanley R Riddell), R01 CA114536, and the Leukemia and Lymphoma Society. Michael Hudecek is a scholar of the German Research Foundation (DFG). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Indolent B-cell malignancies are susceptible to the T-cell mediated graft-versus-tumor effect of allogeneic hematopoietic cell transplant as a consequence of recognition of polymorphic and tumor-associated antigens.
- B-cell malignancies express or overexpress several nonpolymorphic proteins that have limited expression on normal tissues, and can be targeted by tumor-specific T cells present in the endogenous repertoire.
- Gene-transfer techniques can be used to endow T cells with tumor specificity, either through the expression of T-cell receptor genes that recognize tumor-associated peptides displayed on MHC molecules, or through the expression of single-chain antibody fragments that are specific for B-lineage surface molecules.
- Effector T cells derived from central memory precursors exhibit long-term *in vivo* persistence after adoptive transfer, migrate to memory T-cell niches and revert to the memory pool, suggesting that adoptive T-cell therapy for cancer employing central memory cells may have improved efficacy.
- T-cell homing to tumor sites and maintenance of effector function in the immunosuppressive tumor microenvironment will be critical for the success of adoptive T-cell therapy.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Maloney DG. Immunotherapy for non-Hodgkin's lymphoma: monoclonal antibodies and vaccines. *J. Clin. Oncol.* 23(26), 6421–6428 (2005).
- 2 Kay NE, Rai KR, O'Brien S. Chronic lymphocytic leukemia: current and emerging treatment approaches. *Clin. Adv. Hematol. Oncol.* 4(11 Suppl. 22), 1–10 (2006).
- 3 Ravandi F, O'Brien S. Alemtuzumab in CLL and other lymphoid neoplasms. *Cancer Invest.* 24(7), 718–725 (2006).
- 4 Bensinger WI. Role of autologous and allogeneic stem cell transplantation in myeloma. *Leukemia* 23(3), 442–448 (2009).
- 5 Maloney D. Allogeneic transplantation following nonmyeloablative conditioning for aggressive lymphoma. *Bone Marrow Transplant.* 42(Suppl. 1), S35–S36 (2008).

- 6 Rotta M, Storer BE, Sahebi F *et al.* Long-term outcome of patients with multiple myeloma after autologous hematopoietic cell transplantation and nonmyeloablative allografting. *Blood* 113(14), 3383–3391 (2009).
- 7 Sorrow ML, Storer BE, Sandmaier BM *et al.* Five-year follow-up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *J. Clin. Oncol.* 26(30), 4912–4920 (2008).
- Durable complete remissions can be induced following non-myeloablative hematopoietic cell transplantation (HCT) in a subset of patients with advanced chemotherapy-refractory B-cell chronic lymphocytic leukemia (B-CLL) and provides evidence for the susceptibility of B-CLL to the T-cell-mediated graft-versus-tumor (GVT) effect. Also demonstrates that the beneficial GVT effect and detrimental graft-versus-host disease (GVHD) cannot be consistently separated with current approaches and that alternative therapeutic modalities are required to confer anti-tumor reactivity in patients who do not respond to HCT.
- 8 Kessels HW, Wolkers MC, van den Boom MD, van der Valk MA, Schumacher TN. Immunotherapy through TCR gene transfer. *Nat. Immunol.* 2(10), 957–961 (2001).
- 9 Maher J, Brentjens RJ, Gunset G, Riviere I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCR ζ /CD28 receptor. *Nat. Biotechnol.* 20(1), 70–75 (2002).
- 10 Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N. Engl. J. Med.* 257(11), 491–496 (1957).
- 11 Mathe G, Amiel JL, Schwarzenberg L, Cattani A, Schneider M. Adoptive immunotherapy of acute leukemia: experimental and clinical results. *Cancer Res.* 25(9), 1525–1531 (1965).
- 12 Weiden PL, Flournoy N, Thomas ED *et al.* Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N. Engl. J. Med.* 300(19), 1068–1073 (1979).
- 13 Horowitz MM, Gale RP, Sondel PM *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 75(3), 555–562 (1990).
- 14 Bensinger WI, Buckner CD, Anasetti C *et al.* Allogeneic marrow transplantation for multiple myeloma: an analysis of risk factors on outcome. *Blood* 88(7), 2787–2793 (1996).
- 15 Hosing C, Saliba RM, McLaughlin P *et al.* Long-term results favor allogeneic over autologous hematopoietic stem cell transplantation in patients with refractory or recurrent indolent non-Hodgkin's lymphoma. *Ann. Oncol.* 14(5), 737–744 (2003).
- 16 Michallet M, Corront B, Hollard D *et al.* Allogeneic bone marrow transplantation in chronic lymphocytic leukemia: 17 cases. Report from the EBMTG. *Bone Marrow Transplant.* 7(4), 275–279 (1991).
- 17 Pavletic ZS, Arrowsmith ER, Bierman PJ *et al.* Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia. *Bone Marrow Transplant.* 25(7), 717–722 (2000).
- 18 Toze CL, Shepherd JD, Connors JM *et al.* Allogeneic bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia. *Bone Marrow Transplant.* 25(6), 605–612 (2000).
- 19 Kolb HJ, Schattenberg A, Goldman JM *et al.* Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 86(5), 2041–2050 (1995).
- 20 Bethge WA, Hegenbart U, Stuart MJ *et al.* Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood* 103(3), 790–795 (2004).
- 21 Collins RH Jr, Shpilberg O, Drobyski WR *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J. Clin. Oncol.* 15(2), 433–444 (1997).
- 22 Russell NH, Byrne JL, Faulkner RD, Gilyead M, Das-Gupta EP, Haynes AP. Donor lymphocyte infusions can result in sustained remissions in patients with residual or relapsed lymphoid malignancy following allogeneic haemopoietic stem cell transplantation. *Bone Marrow Transplant.* 36(5), 437–441 (2005).
- 23 Choudhury A, Gajewski JL, Liang JC *et al.* Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood* 89(4), 1133–1142 (1997).
- 24 Chalandon Y, Passweg JR, Schmid C *et al.* Outcome of patients developing GVHD after DLI given to treat CML relapse: a study by the chronic leukemia working party of the EBMT. *Bone Marrow Transplant.* DOI: 10.1038/bmt.2009.177 (2009) (Epub ahead of print).
- 25 Mackinnon S, Papadopoulos EB, Carabasi MH *et al.* Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* 86(4), 1261–1268 (1995).
- 26 Simula MP, Marktel S, Fozza C *et al.* Response to donor lymphocyte infusions for chronic myeloid leukemia is dose-dependent: the importance of escalating the cell dose to maximize therapeutic efficacy. *Leukemia* 21(5), 943–948 (2007).
- 27 McSweeney PA, Niederwieser D, Shizuru JA *et al.* Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 97(11), 3390–3400 (2001).
- 28 Niederwieser D, Maris M, Shizuru JA *et al.* Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 101(4), 1620–1629 (2003).
- 29 Maris MB, Niederwieser D, Sandmaier BM *et al.* HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 102(6), 2021–2030 (2003).
- 30 Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat. Rev. Cancer* 4(5), 371–380 (2004).
- 31 den Haan JM, Meadows LM, Wang W *et al.* The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* 279(5353), 1054–1057 (1998).
- First study to describe the molecular basis and the most common mechanism for the generation of minor histocompatibility antigens in humans – a nonsynonymous single nucleotide polymorphism that leads to the binding of peptides to HLA molecules that are recognized as 'non-self' by CD8⁺ T cells from an HLA-identical individual.

- 32 Murata M, Warren EH, Riddell SR. A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J. Exp. Med.* 197(10), 1279–1289 (2003).
- 33 Warren EH, Vigneron NJ, Gavin MA *et al.* An antigen produced by splicing of noncontiguous peptides in the reverse order. *Science* 313(5792), 1444–1447 (2006).
- 34 Nishida T, Hudecek M, Kostic A *et al.* Development of tumor-reactive T cells after nonmyeloablative allogeneic hematopoietic stem cell transplant for chronic lymphocytic leukemia. *Clin. Cancer Res.* 15(14), 4759–4768 (2009) (In press).
- 35 Akatsuka Y, Nishida T, Kondo E *et al.* Identification of a polymorphic gene, BCL2A1, encoding two novel hematopoietic lineage-specific minor histocompatibility antigens. *J. Exp. Med.* 197(11), 1489–1500 (2003).
- 36 de Rijke B, van Horssen-Zoetbrood A, Beekman JM *et al.* A frameshift polymorphism in P2X5 elicits an allogeneic cytotoxic T lymphocyte response associated with remission of chronic myeloid leukemia. *J. Clin. Invest.* 115(12), 3506–3516 (2005).
- 37 Brickner AG, Evans AM, Mito JK *et al.* The *PANE1* gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. *Blood* 107(9), 3779–3786 (2006).
- 38 Dolstra H, de Rijke B, Fredrix H *et al.* Bi-directional allelic recognition of the human minor histocompatibility antigen HB-1 by cytotoxic T lymphocytes. *Eur. J. Immunol.* 32(10), 2748–2758 (2002).
- 39 Spaapen RM, Lokhorst HM, van den Oudenalder K *et al.* Toward targeting B cell cancers with CD4⁺ CTLs: identification of a CD19-encoded minor histocompatibility antigen using a novel genome-wide analysis. *J. Exp. Med.* 205(12), 2863–2872 (2008).
- 40 Spierings E, Hendriks M, Absi L *et al.* Phenotype frequencies of autosomal minor histocompatibility antigens display significant differences among populations. *PLoS Genet.* 3(6), e103 (2007).
- 41 Kamei M, Nannya Y, Torikai H *et al.* HapMap scanning of novel human minor histocompatibility antigens. *Blood* 113(21), 5041–5048 (2008).
- 42 Pinilla-Ibarz J, Cathcart K, Korontsvit T *et al.* Vaccination of patients with chronic myelogenous leukemia with *bcr-abl* oncogene breakpoint fusion peptides generates specific immune responses. *Blood* 95(5), 1781–1787 (2000).
- 43 Davis TA, Maloney DG, Czerwinski DK, Liles TM, Levy R. Anti-idiotypic antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. *Blood* 92(4), 1184–1190 (1998).
- 44 Meeker T, Lowder J, Cleary ML *et al.* Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotypic antibodies. *N. Engl. J. Med.* 312(26), 1658–1665 (1985).
- 45 Bogen B, Malissen B, Haas W. Idiotope-specific T cell clones that recognize syngeneic immunoglobulin fragments in the context of class II molecules. *Eur. J. Immunol.* 16(11), 1373–1378 (1986).
- 46 Kwak LW, Campbell MJ, Czerwinski DK, Hart S, Miller RA, Levy R. Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N. Engl. J. Med.* 327(17), 1209–1215 (1992).
- **First clinical trial of immunoglobulin idiotype vaccination showing induction of idiotype-specific humoral and cellular immune responses in patients with follicular lymphoma after active immunization with autologous idiotype protein.**
- 47 Timmerman JM, Czerwinski DK, Davis TA *et al.* Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. *Blood* 99(5), 1517–1526 (2002).
- 48 Bendandi M, Gocke CD, Kobrin CB *et al.* Complete molecular remissions induced by patient-specific vaccination plus granulocyte–monocyte colony-stimulating factor against lymphoma. *Nat. Med.* 5(10), 1171–1177 (1999).
- 49 Inoges S, Rodriguez-Calvillo M, Zabalegui N *et al.* Clinical benefit associated with idiotype vaccination in patients with follicular lymphoma. *J. Natl Cancer Inst.* 98(18), 1292–1301 (2006).
- 50 Ai WZ, Tibshirani R, Taidi B, Czerwinski D, Levy R. Anti-idiotypic antibody response after vaccination correlates with better overall survival in follicular lymphoma. *Blood* 113(23), 5743–5746 (2009).
- 51 Baskar S, Kobrin CB, Kwak LW. Autologous lymphoma vaccines induce human T cell responses against multiple, unique epitopes. *J. Clin. Invest.* 113(10), 1498–1510 (2004).
- 52 Trojan A, Schultze JL, Witzens M *et al.* Immunoglobulin framework-derived peptides function as cytotoxic T-cell epitopes commonly expressed in B-cell malignancies. *Nat. Med.* 6(6), 667–672 (2000).
- 53 Houot R, Levy R. Vaccines for lymphomas: idiotype vaccines and beyond. *Blood Rev.* 23(3), 137–142 (2009).
- 54 Osterborg A, Yi Q, Henriksson L *et al.* Idiotype immunization combined with granulocyte–macrophage colony-stimulating factor in myeloma patients induced type I, major histocompatibility complex-restricted, CD8- and CD4-specific T-cell responses. *Blood* 91(7), 2459–2466 (1998).
- 55 Wen YJ, Ling M, Bailey-Wood R, Lim SH. Idiotype protein-pulsed adherent peripheral blood mononuclear cell-derived dendritic cells prime immune system in multiple myeloma. *Clin. Cancer Res.* 4(4), 957–962 (1998).
- 56 Brown RD, Pope B, Murray A *et al.* Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor- β 1 and interleukin-10. *Blood* 98(10), 2992–2998 (2001).
- 57 Bogen B, Schenck K, Munthe LA, Dembic Z. Deletion of idiotype (Id)-specific T cells in multiple myeloma. *Acta Oncol.* 39(7), 783–788 (2000).
- 58 Hong S, Qian J, Yang J, Li H, Kwak LW, Yi Q. Roles of idiotype-specific T cells in myeloma cell growth and survival: Th1 and CTL cells are tumoricidal while Th2 cells promote tumor growth. *Cancer Res.* 68(20), 8456–8464 (2008).
- 59 Osterroth F, Garbe A, Fisch P, Veelken H. Stimulation of cytotoxic T cells against idiotype immunoglobulin of malignant lymphoma with protein-pulsed or idiotype-transduced dendritic cells. *Blood* 95(4), 1342–1349 (2000).
- 60 Vera J, Savoldo B, Vigouroux S *et al.* T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. *Blood* 108(12), 3890–3897 (2006).
- 61 Di Nicola M, Zappasodi R, Carlo-Stella C *et al.* Vaccination with autologous tumor-loaded dendritic cells induces clinical and immunologic responses in indolent B-cell lymphoma patients with relapsed and measurable disease: a pilot study. *Blood* 113(1), 18–27 (2009).

- 62 Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1(7335), 702–703 (1964).
- 63 Papadopoulos EB, Ladanyi M, Emanuel D *et al.* Infusions of donor leukocytes to treat Epstein–Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N. Engl. J. Med.* 330(17), 1185–1191 (1994).
- 64 Rooney CM, Smith CA, Ng CY *et al.* Infusion of cytotoxic T cells for the prevention and treatment of Epstein–Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* 92(5), 1549–1555 (1998).
- 65 Blake N, Lee S, Redchenko I *et al.* Human CD8⁺ T cell responses to EBV EBNA1: HLA class I presentation of the (Gly-Ala)-containing protein requires exogenous processing. *Immunity* 7(6), 791–802 (1997).
- 66 Yin Y, Manoury B, Fahraeus R. Self-inhibition of synthesis and antigen presentation by Epstein–Barr virus-encoded EBNA1. *Science* 301(5638), 1371–1374 (2003).
- 67 Bollard CM, Gottschalk S, Huls MH *et al.* *In vivo* expansion of LMP 1- and 2-specific T-cells in a patient who received donor-derived EBV-specific T-cells after allogeneic stem cell transplantation. *Leuk. Lymphoma* 47(5), 837–842 (2006).
- 68 Bollard CM, Gottschalk S, Leen AM *et al.* Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. *Blood* 110(8), 2838–2845 (2007).
- 69 Bollard CM, Aguilar L, Straathof KC *et al.* Cytotoxic T lymphocyte therapy for Epstein–Barr virus+ Hodgkin's disease. *J. Exp. Med.* 200(12), 1623–1633 (2004).
- **Demonstrates that the adoptive transfer of Epstein–Barr virus (EBV)-reactive T cells to patients with Hodgkin disease can augment CD8⁺ T cell immunity to the weakly immunogenic LMP-2 protein, and that the infused T cells migrate to tumor sites and promote tumor regression.**
- 70 Klein U, Tu Y, Stolovitzky GA *et al.* Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J. Exp. Med.* 194(11), 1625–1638 (2001).
- 71 Jelinek DF, Tschumper RC, Stolovitzky GA *et al.* Identification of a global gene expression signature of B-chronic lymphocytic leukemia. *Mol. Cancer Res.* 1(5), 346–361 (2003).
- 72 Shaughnessy J Jr, Zhan F, Barlogie B, Stewart AK. Gene expression profiling and multiple myeloma. *Best Pract. Res. Clin. Haematol.* 18(4), 537–552 (2005).
- 73 Mayr C, Bund D, Schlee M *et al.* MDM2 is recognized as a tumor-associated antigen in chronic lymphocytic leukemia by CD8⁺ autologous T lymphocytes. *Exp. Hematol.* 34(1), 44–53 (2006).
- 74 Mayr C, Bund D, Schlee M *et al.* Fibromodulin as a novel tumor-associated antigen (TAA) in chronic lymphocytic leukemia (CLL), which allows expansion of specific CD8⁺ autologous T lymphocytes. *Blood* 105(4), 1566–1573 (2005).
- 75 Schmidt SM, Schag K, Muller MR *et al.* Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. *Blood* 102(2), 571–576 (2003).
- 76 Frank C, Hundemer M, Ho AD, Goldschmidt H, Witzens-Harig M. Cellular immune responses against the cancer-testis antigen SPAN-XB in healthy donors and patients with multiple myeloma. *Leuk. Lymphoma* 49(4), 779–785 (2008).
- 77 Hundemer M, Schmidt S, Condomines M *et al.* Identification of a new HLA-A2-restricted T-cell epitope within HM1.24 as immunotherapy target for multiple myeloma. *Exp. Hematol.* 34(4), 486–496 (2006).
- 78 Qian J, Xie J, Hong S *et al.* Dickkopf-1 (DKK1) is a widely expressed and potent tumor-associated antigen in multiple myeloma. *Blood* 110(5), 1587–1594 (2007).
- 79 Hatta Y, Takeuchi J, Saitoh T *et al.* WT1 expression level and clinical factors in multiple myeloma. *J. Exp. Clin. Cancer Res.* 24(4), 595–599 (2005).
- 80 van Baren N, Brasseur F, Godelaine D *et al.* Genes encoding tumor-specific antigens are expressed in human myeloma cells. *Blood* 94(4), 1156–1164 (1999).
- 81 Menssen HD, Renkl HJ, Rodeck U *et al.* Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia* 9(6), 1060–1067 (1995).
- 82 Doubrovina ES, Doubrovin MM, Lee S *et al.* *In vitro* stimulation with WT1 peptide-loaded Epstein–Barr virus-positive B cells elicits high frequencies of WT1 peptide-specific T cells with *in vitro* and *in vivo* tumoricidal activity. *Clin. Cancer Res.* 10(21), 7207–7219 (2004).
- 83 Ho WY, Nguyen HN, Wolf M, Kuball J, Greenberg PD. *In vitro* methods for generating CD8⁺ T-cell clones for immunotherapy from the naive repertoire. *J. Immunol. Methods* 310(1–2), 40–52 (2006).
- 84 Rezvani K, Yong AS, Savani BN *et al.* Graft-versus-leukemia effects associated with detectable Wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. *Blood* 110(6), 1924–1932 (2007).
- 85 Tsuboi A, Oka Y, Nakajima H *et al.* Wilms tumor gene WT1 peptide-based immunotherapy induced a minimal response in a patient with advanced therapy-resistant multiple myeloma. *Int. J. Hematol.* 86(5), 414–417 (2007).
- 86 Goodyear O, Piper K, Khan N *et al.* CD8⁺ T cells specific for cancer germline gene antigens are found in many patients with multiple myeloma, and their frequency correlates with disease burden. *Blood* 106(13), 4217–4224 (2005).
- 87 van Rhee F, Szmania SM, Zhan F *et al.* NY-ESO-1 is highly expressed in poor-prognosis multiple myeloma and induces spontaneous humoral and cellular immune responses. *Blood* 105(10), 3939–3944 (2005).
- 88 Hunder NN, Wallen H, Cao J *et al.* Treatment of metastatic melanoma with autologous CD4⁺ T cells against NY-ESO-1. *N. Engl. J. Med.* 358(25), 2698–2703 (2008).
- **Describes the regression of melanoma metastases following adoptive transfer of an autologous CD4⁺ T-cell clone specific for the NY-ESO-1 antigen and underlines the potential use of CD4⁺ T cells in adoptive T-cell therapy for human malignancies.**
- 89 Atanackovic D, Luetkens T, Hildebrandt Y *et al.* Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma. *Clin. Cancer Res.* 15(4), 1343–1352 (2009).
- 90 Dembic Z, Haas W, Weiss S *et al.* Transfer of specificity by murine α and β T-cell receptor genes. *Nature* 320(6059), 232–238 (1986).
- 91 Mommaas B, van Halteren AG, Pool J *et al.* Adult and cord blood T cells can acquire HA-1 specificity through HA-1 T-cell receptor gene transfer. *Haematologica* 90(10), 1415–1421 (2005).
- 92 Morgan RA, Dudley ME, Wunderlich JR *et al.* Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 314(5796), 126–129 (2006).

- First study using genetically engineered autologous T cells to express a tumor-reactive T cell receptor (TCR) by retrovirus-mediated gene transfer. The study showed tumor regression in a small fraction of patients with metastatic melanoma and revealed several challenges associated with this approach including the requirement for high levels of persistent TCR transgene expression.
- 93 Stanislawski T, Voss RH, Lotz C *et al.* Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat. Immunol.* 2(10), 962–970 (2001).
- 94 Zhao Y, Zheng Z, Robbins PF, Khong HT, Rosenberg SA, Morgan RA. Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines. *J. Immunol.* 174(7), 4415–4423 (2005).
- 95 Engels B, Cam H, Schuler T *et al.* Retroviral vectors for high-level transgene expression in T lymphocytes. *Hum. Gene Ther.* 14(12), 1155–1168 (2003).
- 96 Yang S, Cohen CJ, Peng PD *et al.* Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition. *Gene Ther.* 15(21), 1411–1423 (2008).
- 97 Costello E, Munoz M, Buetti E, Meylan PR, Diggelmann H, Thali M. Gene transfer into stimulated and unstimulated T lymphocytes by HIV-1-derived lentiviral vectors. *Gene Ther.* 7(7), 596–604 (2000).
- 98 Cavalieri S, Cazzaniga S, Geuna M *et al.* Human T lymphocytes transduced by lentiviral vectors in the absence of TCR activation maintain an intact immune competence. *Blood* 102(2), 497–505 (2003).
- 99 Wu X, Li Y, Crise B, Burgess SM. Transcription start regions in the human genome are favored targets for MLV integration. *Science* 300(5626), 1749–1751 (2003).
- 100 Schroder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 110(4), 521–529 (2002).
- 101 Howe SJ, Mansour MR, Schwarzwaelder K *et al.* Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* 118(9), 3143–3150 (2008).
- Reports the activation of an oncogene as a consequence of γ -retroviral vector integration and occurrence of clonal T-cell proliferation in a clinical gene therapy trial for X-linked SCID.
- 102 Thornhill SI, Schambach A, Howe SJ *et al.* Self-inactivating gammaretroviral vectors for gene therapy of X-linked severe combined immunodeficiency. *Mol. Ther.* 16(3), 590–598 (2008).
- 103 Montini E, Cesana D, Schmidt M *et al.* The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *J. Clin. Invest.* 119(4), 964–975 (2009).
- 104 Newrzela S, Cornils K, Li Z *et al.* Resistance of mature T cells to oncogene transformation. *Blood* 112(6), 2278–2286 (2008).
- 105 Jensen MC, Clarke P, Tan G *et al.* Human T lymphocyte genetic modification with naked DNA. *Mol. Ther.* 1(1), 49–55 (2000).
- 106 Schaft N, Dorrie J, Muller I *et al.* A new way to generate cytolytic tumor-specific T cells: electroporation of RNA coding for a T cell receptor into T lymphocytes. *Cancer Immunol. Immunother.* 55(9), 1132–1141 (2006).
- 107 Ivics Z, Hackett PB, Plasterk RH, Izsvak Z. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. *Cell* 91(4), 501–510 (1997).
- 108 Huang X, Wilber AC, Bao L *et al.* Stable gene transfer and expression in human primary T cells by the Sleeping Beauty transposon system. *Blood* 107(2), 483–491 (2006).
- 109 Kuball J, Dossett ML, Wolff M *et al.* Facilitating matched pairing and expression of TCR chains introduced into human T cells. *Blood* 109(6), 2331–2338 (2007).
- 110 van der Veken LT, Coccoris M, Swart E, Falkenburg JH, Schumacher TN, Heemskerk MH. $\alpha\beta$ T cell receptor transfer to $\gamma\delta$ T cells generates functional effector cells without mixed TCR dimers *in vivo*. *J. Immunol.* 182(1), 164–170 (2009).
- 111 Li Y, Moysey R, Molloy PE *et al.* Directed evolution of human T-cell receptors with picomolar affinities by phage display. *Nat. Biotechnol.* 23(3), 349–354 (2005).
- 112 Hiddemann W, Kneba M, Dreyling M *et al.* Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 106(12), 3725–3732 (2005).
- 113 Wierda W, O'Brien S, Wen S *et al.* Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. *J. Clin. Oncol.* 23(18), 4070–4078 (2005).
- 114 Schumacher TN. T-cell-receptor gene therapy. *Nat. Rev. Immunol.* 2(7), 512–519 (2002).
- 115 Kowolik CM, Topp MS, Gonzalez S *et al.* CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances *in vivo* persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res.* 66(22), 10995–11004 (2006).
- 116 Till BG, Jensen MC, Wang J *et al.* Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 112(6), 2261–2271 (2008).
- 117 Cooper LJ, Topp MS, Serrano LM *et al.* T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. *Blood* 101(4), 1637–1644 (2003).
- 118 James SE, Greenberg PD, Jensen MC *et al.* Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane. *J. Immunol.* 180(10), 7028–7038 (2008).
- 119 Ochsenbein AF, Sierro S, Odermatt B *et al.* Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. *Nature* 411(6841), 1058–1064 (2001).
- 120 Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure – an obstacle in cancer therapy. *Nat. Rev. Cancer* 4(10), 806–813 (2004).
- 121 Weishaupt C, Munoz KN, Buzney E, Kupper TS, Fuhlbrigge RC. T-cell distribution and adhesion receptor expression in metastatic melanoma. *Clin. Cancer Res.* 13(9), 2549–2556 (2007).
- 122 Kershaw MH, Wang G, Westwood JA *et al.* Redirecting migration of T cells to chemokine secreted from tumors by genetic modification with CXCR2. *Hum. Gene Ther.* 13(16), 1971–1980 (2002).

- 123 Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc. Natl Acad. Sci. USA* 99(19), 12293–12297 (2002).
- 124 Yamamoto R, Nishikori M, Kitawaki T *et al.* PD-1–PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. *Blood* 111(6), 3220–3224 (2008).
- 125 Foster AE, Dotti G, Lu A *et al.* Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF- β receptor. *J. Immunother.* 31(5), 500–505 (2008).
- 126 Rimsza LM, Roberts RA, Miller TP *et al.* Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. *Blood* 103(11), 4251–4258 (2004).
- 127 Gorgun G, Holderried TA, Zahrieh D, Neuberg D, Gribben JG. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. *J. Clin. Invest.* 115(7), 1797–1805 (2005).
- 128 Ramsay AG, Johnson AJ, Lee AM *et al.* Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. *J. Clin. Invest.* 118(7), 2427–2437 (2008).
- Demonstrates that B-CLL cells form deficient immune synapses with autologous and allogeneic T cells from tumor-bearing patients to escape immune recognition. The formation of functional immune synapses could be restored after treatment with the immunomodulatory drug lenalidomide. These findings illustrate the need to overcome mechanisms employed by malignant cells that lead to suppression of T-cell effector function in the tumor environment.
- 129 Ferrajoli A, Lee BN, Schlette EJ *et al.* Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood* 111(11), 5291–5297 (2008).
- 130 Yee C, Thompson JA, Byrd D *et al.* Adoptive T cell therapy using antigen-specific CD8⁺ T cell clones for the treatment of patients with metastatic melanoma: *in vivo* persistence, migration, and antitumor effect of transferred T cells. *Proc. Natl Acad. Sci. USA* 99(25), 16168–16173 (2002).
- 131 Baluna R, Vitetta ES. Vascular leak syndrome: a side effect of immunotherapy. *Immunopharmacology* 37(2–3), 117–132 (1997).
- 132 Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. *Nat. Immunol.* 1(5), 426–432 (2000).
- 133 Schluns KS, Williams K, Ma A, Zheng XX, Lefrancois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. *J. Immunol.* 168(10), 4827–4831 (2002).
- 134 Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8⁺ cells but are not required for memory phenotype CD4⁺ cells. *J. Exp. Med.* 195(12), 1523–1532 (2002).
- 135 Dudley ME, Yang JC, Sherry R *et al.* Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J. Clin. Oncol.* 26(32), 5233–5239 (2008).
- Autologous tumor-infiltrating lymphocytes from patients with metastatic melanoma were expanded *in vitro* and reinfused into patients after administration of lymphodepleting preparative regimens. Host lymphodepletion was associated with increased serum levels of the homeostatic lymphocyte cytokines IL-7 and IL-15 and the objective response rate was superior to historic studies where tumor-infiltrating lymphocytes were administered without lymphodepleting chemotherapy.
- 136 Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* 257(5067), 238–241 (1992).
- 137 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401(6754), 708–712 (1999).
- 138 Lanzavecchia A, Sallusto F. Understanding the generation and function of memory T cell subsets. *Curr. Opin. Immunol.* 17(3), 326–332 (2005).
- 139 Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8⁺ T cells derived from central memory cells establishes persistent T cell memory in primates. *J. Clin. Invest.* 118(1), 294–305 (2008).
- Tracked the fate of clonally derived effector T cells isolated from either central memory and effector memory precursors in a nonhuman primate model. Effector T cells derived from central memory precursors persisted long-term after adoptive transfer, required memory phenotype and reverted to both central and effector memory cells whereas effector memory-derived T-cell clones rapidly underwent apoptosis. These findings suggest that deriving tumor antigen-specific T cells from central memory precursors will provide a cell population with superior intrinsic capacity to persist after adoptive transfer.
- 140 Savoldo B, Rooney CM, Di Stasi A *et al.* Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30 ζ artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. *Blood* 110(7), 2620–2630 (2007).
- 141 Pule MA, Savoldo B, Myers GD *et al.* Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat. Med.* 14(11), 1264–1270 (2008).
- 142 Berger C, Blau CA, Huang ML *et al.* Pharmacologically regulated Fas-mediated death of adoptively transferred T cells in a nonhuman primate model. *Blood* 103(4), 1261–1269 (2004).
- 143 Kieback E, Charo J, Sommermeyer D, Blankenstein T, Uckert W. A safeguard eliminates T cell receptor gene-modified autoreactive T cells after adoptive transfer. *Proc. Natl Acad. Sci. USA* 105(2), 623–628 (2008).
- 144 Reff ME, Carner K, Chambers KS *et al.* Depletion of B cells *in vivo* by a chimeric mouse human monoclonal antibody to CD20. *Blood* 83(2), 435–445 (1994).
- 145 Tiberghien P, Reynolds CW, Keller J *et al.* Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: an approach for specific *in vivo* donor T-cell depletion after bone marrow transplantation? *Blood* 84(4), 1333–1341 (1994).
- 146 Fearon DT, Manders P, Wagner SD. Arrested differentiation, the self-renewing memory lymphocyte, and vaccination. *Science* 293(5528), 248–250 (2001).

- 147 Chang JT, Palanivel VR, Kinjyo I *et al.* Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* 315(5819), 1687–1691 (2007).
- 148 Zhang Y, Joe G, Hexner E, Zhu J, Emerson SG. Host-reactive CD8⁺ memory stem cells in graft-versus-host disease. *Nat. Med.* 11(12), 1299–1305 (2005).
- 149 Robertson LE, Plunkett W, McConnell K, Keating MJ, McDonnell TJ. Bcl-2 expression in chronic lymphocytic leukemia and its correlation with the induction of apoptosis and clinical outcome. *Leukemia* 10(3), 456–459 (1996).
- 150 Giannopoulos K, Li L, Bojarska-Junak A *et al.* Expression of RHAMM/CD168 and other tumor-associated antigens in patients with B-cell chronic lymphocytic leukemia. *Int. J. Oncol.* 29(1), 95–103 (2006).

Affiliations

- Michael Hudecek
Clinical Research Division, Fred
Hutchinson Cancer Research Center,
Seattle, WA, USA
and
Department of Hematology, Oncology and
Coagulation, University of Leipzig Medical
Center, University of Leipzig, Germany
Tel.: +1 206 667 4181
Fax: +1 206 667 7983
mhudecek@fhcrc.org
- Larry D Anderson Jr
University of Texas Southwestern Medical
Center, Department of Internal Medicine,
Hematology–Oncology, 5323 Harry Hines
Blvd, Dallas, TX 75390, USA
Tel.: +1 214 648 5906
Fax: +1 214 648 4152
larry.anderson@utsouthwestern.edu

- Tetsuya Nishida
Nagoya University Graduate School of
Medicine, 65-Tsurumai-cho, Showa-ku,
Nagoya, Aichi 466-8560, Japan
Tel.: +81 527 442 145
Fax: +81 527 442161
tnishida@med.nagoya-u.ac.jp
- Stanley R Riddell, MD
Departments of Medicine, University of
Washington, Seattle, WA, USA
and
Clinical Research Division,
Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue N, D3-100, Seattle,
WA 98109, USA
Tel.: +1 206 667 5249
Fax: +1 206 667 7983
sriddell@fhcrc.org