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Summary

The graft-*versus*-leukaemia (GVL) effect following allogeneic stem cell transplantation is clear evidence that T lymphocytes can control and eliminate myeloid leukaemias. The successful identification of a range of leukaemia specific antigens (LSA) in recent years has stimulated efforts to induce leukaemia specific T cell responses to these antigens with peptide vaccines. An ideal LSA should be restricted in its expression to leukaemia including progenitor cells, intrinsically connected with the leukaemic phenotype, and capable of inducing strong cytotoxic T cell responses to the leukaemia. Peptides from three well-characterized LSA, the breakpoint cluster region–abelson (BCR–ABL) fusion protein of chronic myelogenous leukaemia, proteinase-3 and Wilms tumour 1 protein, serve as the basis for several clinical trials using peptide and adjuvants to treat patients with a variety of myeloid malignancies. Preliminary results from these studies indicate that these peptides induce immune responses which can translate into clinical responses which include complete remissions from leukaemia. These promising early results point the way to optimizing the administration of peptide vaccines and suggest ways of combining vaccination with allogeneic stem cell transplantation to boost GVL effects.

Keywords: clinical trials, myeloid leukaemia, peptide, vaccines

Introduction

The idea of using vaccines to treat myeloid leukaemia is not new. In the 1970s several investigators treated acute myeloid leukaemia (AML) patients entering remission with vaccines combining bacille Calmette–Guérin (BCG) with irradiated leukaemia cells. Enthusiasm for the approach waned as it became clear that vaccine recipients had no survival advantage [1]. Clinical interest in immunotherapy for myeloid leukaemias turned towards allogeneic stem cell transplantation (SCT), where it became increasingly clear that the transplant conferred a powerful T cell-mediated graft-*versus*-leukaemia (GVL) effect. The potential of allogeneic donor lymphocyte infusions to induce remissions in patients relapsing after SCT was strong evidence that T cells could destroy leukaemia [2–5]. This realization led to efforts using *in vitro*-generated, leukaemia-specific T cells transferred adoptively to SCT recipients to treat relapsing leukaemia. Despite a successful demonstration of proof of principle, however, the approach has not been applied widely because of its impracticability [6]. Research into cellular vaccines was re-enthused with techniques to generate dendritic cells from AML and chronic myeloid leukaemia (CML) cells. Such dendritic cell vaccines have the potential to stimulate optimally T cell responses to the leukaemia [7–13]. Meanwhile, other groups have begun to re-explore cellular vaccines in the form of leukaemia cell lysates or heat-shock protein fractions from leukaemia cells [14–17]. While these approaches may ultimately prove effective, they represent costly, patient-tailored techniques with variable reproducibility. For these reasons, this review focuses on efforts to discover and use defined leukaemia protein

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Table 1. Candidate proteins for peptide vaccination in myeloid leukaemias.

Antigenic protein	Peptides under development	HLA restriction	Clinical trials	Ref.
Leukaemia-specific antigens				
BCR-ABL	b3a2 fusion region 9–25 mers	HLA-A3,-11, -B-8, DR-1, -4, -11	CML	[53–55]
Primary granule proteins	PR1	A-2	MDS, AML, CML	[72]
	PR7	A-2	No	[102]
PML-RARA	BCR 1–25	DR-11	No	[103]
Tumour-specific antigens				
Wilms tumour-1	WT1-126	A-2	MDS, AML	[36,38]
	WT1-235	A-2, -24	MDS, AML	[38,104]
	WT1-187	A-2	No	[38]
	WT1-37	A-2	No	[105]
H-TERT		A-2, -3, -24	No	[106–108]
PRAME		A-2	No	[109]
BCL-2		A-2	No	[108]
G-250		A-2	No	[108,110]
mHags				
HA-1 protein		A-2	No	[111]
HA-2 protein		A-2		[111]
CD33 allele		A-2	No	[111,112]
CD45 allele		A-2	No	[113]

BCR–ABL: breakpoint cluster region–abelson; WT1: Wilms tumour 1; MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia ; CML: chronic myeloid leukaemia; HLA: human leucocyte antigen.

antigens for immunotherapy, because of their potential to establish universal and practical vaccines for myeloid leukaemias. In the last decade a variety of proteins giving rise to leukaemia-specific antigenic peptides have been characterized and several clinical peptide-based vaccine trials have been initiated in AML and CML and myelodysplastic syndrome (MDS).

Leukaemia-specific antigens

Antigen discovery

The identification of antigenic proteins capable of inducing cytotoxic T cell (CTL) responses against myeloid leukaemias was facilitated greatly by advances in basic immunology, which defined the molecular nature of the interaction of the T cell receptor with peptide antigens presented by major histocompatibility complex locus (MHC) molecules on target cells. At the same time the mapping of intracellular antigen-processing pathways gave us, for the first time, a complete picture of the way in which proteins within the cell are digested by the proteasome into short peptide fragments which enter the endoplasmic reticulum to become assembled into MHC class I/peptide complexes that are then transported to the cell surface for the scrutiny of T cells [18–20]. This revelation led almost immediately to speculation that 'neoantigen' peptide fragments spanning the breakpoints of the leukaemia-specific breakpoint cluster region–abelson (BCR–ABL) fusion protein specific for CML could be incorporated into MHC molecules, presented on the cell surface and be recognized as foreign by cytotoxic T cells [21–24]. The same peptides, it was argued, could be used as a vaccine to treat CML. In the last 15 years or so numerous leukaemia antigens inducing T cell responses have been described. They can be categorized broadly according to whether they are overexpressed or aberrantly expressed self proteins, or neoantigens derived from leukaemia-specific chromosomal recombinations, or alleleic polymorphisms (minor histocompatibility antigens: mHag) which are novel to a stem cell donor lacking that allele (Table 1). Two distinct approaches have been used to identify leukaemia-specific and tumour-specific antigens (LSA and TSA). The 'classical' approach, developed by Boon and colleagues, has been used to characterize a long list of TSA – of interest to haematologists because many TSA are also overexpressed by leukaemias [25,26]. The approach requires the purification and expansion of tumour-specific T cells, typically from tumour-infiltrating lymphocytes. A DNA library from the tumour transfected into antigen-presenting cells (APC) bearing a single human leucocyte antigen (HLA) restriction element is used to generate a series of cell pools containing parts of the library. The pools are screened for cytokine production [interferon (IFN)-γ or tumour necrosis factor (TNF)] by the T cell clone. Only APC-presenting peptides derived from the tumour antigen elicit T cell responses. In a stepwise procedure, the DNA fragments eliciting T cell responses can be narrowed to the precise fragment giving rise to the protein antigen, which can then be identified by database searching. With appropriate DNA fragmentation of the protein sequence, the antigenic peptides derived from the protein can then be defined. Another variation (used successfully to identify the mHag HA-2) is to generate a T

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cell clone by stimulating post-transplant (donor) T cells with cells from the patient. The clone is used to screen peptides eluted from MHC molecules of the target cell and separated by mass spectrometry into peptide fractions [27,28]. The alternative approach (termed 'reverse immunology') does not require the generation of a tumour-specific T cell clone. Instead, a search is made of known proteins with promising antigenic characteristics. Peptides derived from these proteins and predicted to bind to common HLA types are used to generate cytotoxic T cells which are then tested against leukaemia and normal targets to define HLA restriction, leukaemia cytotoxicity and specificity [29–31].

Validation of LSA

Candidate peptides are validated for their applicability as vaccines using a cell panel to test HLA-restriction, leukaemia specificity and ability to induce cytotoxic T cells. Tissue libraries are screened for mRNA expression of the LSA to confirm leukaemia specificity. The anti-leukaemic potential of peptide-specific T cell clones is defined *in vitro* by their cytotoxicity against leukaemic progenitors, with colony and proliferation inhibition assays, or *in vivo* in non-obese diabetic (NOD)–SCID mice inoculated with human leukaemia. Alternatively, specific cytotoxicity against human leukaemic progenitors can be tested by direct cytotoxicity or by inhibition of proliferation [32,33].

Characteristics of an ideal LSA

With an abundance of already defined antigens as well as a growing list of candidate proteins, it is useful to define some criteria for selecting proteins most likely to serve as good LSA (summarized in Table 1).

Leukaemia restriction

Concern that vaccines for LSA also expressed by other tissues might cause autoimmune disease requires a comprehensive description of LSA tissue expression. Fusion proteins arising from specific chromosomal recombinations and linked uniquely to the leukaemic process are safe because they are restricted entirely to leukaemia cells. However, the requirement for exclusive expression by the leukaemia are not absolute: the cancer-testis antigen WT1, expressed on stem cells and gonadal cells, behaves in a leukaemia-restricted manner because only the malignant cells which overexpress WT1 are susceptible targets of T cell attack [34–38]. In the context of SCT only broad graft-*versus*-marrow specificity is required for safety, as the eradicated recipient haematopoiesis is replaced by that of the donor lacking the mHag. There has been concern (so far unjustified) that immune reactions against proteins such as PR3 and NE may target not only the leukaemia cells which overexpress the protein, but also normal tissues that express lesser amounts of the protein such as normal early myeloid precursors. In addition, PR3 is a target of autoimmune attack in the disease Wegener's granulomatosis, a vasculitis associated with the production of IgG1 anti-neutrophil cytoplasmic antibodies (ANCA) with specificity for PR3. This has led to excluding patients with ANCA or a history of vasculitis from vaccination with PR1.

Stem cell expression

There is an assumption that effective immunotherapy requires the target antigen to be expressed by the earliest leukaemic progenitor. While this seems to be a reasonable criterion for antigen selection, it may not be a prerequisite in every case: individual leukaemias have diverse hierarchical patterns of progenitor and progeny. CML progression, for example, appears to derive from the committed granulocytic progenitor [39].

Functional importance for the leukaemic phenotype

Malignant cells have a predilection to escape immune control by down-regulating antigen expression. Selecting antigenic targets that are intrinsic to the maintenance of the leukaemic phenotype (e.g. BCR–ABL, WT1, PR3) may render it impossible for the leukaemia cell to escape and still retain leukaemic function [40–43]. It should be remembered, however, that malignant cells can also escape T cell control by down-regulating MHC molecules allowing them to conserve essential leukaemic features.

Immune response

To be effective as vaccines LSA must elicit clonal expansions of cytotoxic T cells with high avidity for the antigen. Furthermore, such antigen-specific T cells should be validated for their anti-leukaemic effects as described above.

Global applicability

The broad expression of antigens such as WT1 by many leukaemias and solid tumours makes it an attractive protein for peptide manufacture. Similarly, proteins which encode for multiple antigenic peptides binding to multiple class I and II HLA molecules are attractive because this removes the constraints of HLA-restricted vaccine approaches.

Peptide vaccines under clinical development (Table 2)

BCR–ABL vaccine

Four fusion variants of the BCR–ABL protein are known. Of these, the p210 b3a2 variant appears most promising in terms of HLA restriction with many HLA class I and II

Table 2 Peptide vaccines under clinical evaluation in myeloid malignancies.

Characteristic	BCR–ABL	PR1	WT1
Expression	Unique to CML	Myeloid malignancies	Many leukaemias and solid tumours
Essential for phenotype	Yes	Probably	Yes
Peptide(s)/HLA restriction	SSKALQRPV/A-2, KQSSKALQR/A-3 ATGFKQSSK/A-11 HSATGFKQSSK/A-3,A-11 GFKQSSKAL B-8 IVHSATGFKQSSKALQRP VASDFEP /DR-1, -4, -11	VLQENTVLV/A-2	RMFPNAPYL/A-2 CMTWNQMNL /A-24 CYTWNQMNL (heteroclitic)/A-24
Size (mer)	9,11,15	9	9
Adjuvant	QS-21 (*Quillaja saponaria*) GM-CSF	Montanide GM-CSF	Montanide GM-CSF
In vitro-generated CTL kill leukaemia cells	Variable	+ + +	+ + +
Affinity of T cells to peptide	Low	High and low	High and low
Natural T cell immunity			
In controls	+/–	< 1/100 000	< 1/100 000
In leukaemia	+/–	< 1/10 000	1/10 000
After SCT	May be lytic to CML	Up to 13% CD8	Up to 5% CD8
Antibodies identified	Yes (after vaccination)	Yes (ANCA)	Yes (in leukaemia and MDS patients)
T cell response to vaccine	+	+	+
Clinical responses to vaccine	+	+	+

BCR–ABL: breakpoint cluster region–abelson; WT1: Wilms tumour 1; MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia ; CML: chronic myeloid leukaemia; HLA: human leucocyte antigen; PR1: proteinase 1; CTL: cytotoxic T cell; GM-CSF: granulocyte–macrophage colony-stimulating factor; ANCA: anti-neutrophil cytoplasmic antibodies.

binding peptides [21,23,24,44–47]. Recently, Melief and colleagues identified 17 novel, potentially antigenic, BCR–ABL fusion peptides [48]. Ten HLA class I binding fusion peptides fulfilled the essential requirement for antigen presentation of being excised after their C-terminus by the proteasome. It is clear from numerous descriptions that b3a2 peptides can induce peptide-specific T cells and that CML cells do present BCR–ABL peptides, but BCR–ABL peptides have not been found reproducibly to induce CTL lytic for CML. The most convincing results come from studies by Clark *et al*., who were able to generate MHC-restricted CTL responses to CML cells using an HLA A-3 binding peptide [49]. *In vivo* peptide-specific CTL have been identified after stem cell transplant in patients with controlled CML and in HLA-A3 positive healthy individuals [49–52]. These cells had low affinity for the peptide with variable lytic activity against fresh CML targets.

BCR–ABL trials

Pinilla-lbarz and colleagues, at the Memorial Sloane Kettering Institute, were the first to develop a BCR–ABL vaccine composed of a pool of six peptide fragments (Table 2). A safety study showed that the vaccine and adjuvant were well tolerated and could elicit T cell immune responses to BCR–ABL [53]. They then performed a phase II study where 14 CML patients in chronic phase received five doses of the vaccine and adjuvant [54]. All patients developed delayed-type hypersensitivity (DTH) or $CD4^+$ proliferative responses to the peptides. IFN-γ-producing $CD4^+$ and $CD8^+$ T cells developed in 11 and four patients, respectively. Reduction in marrow Ph chromosomes occurred in four patients, but three of these continued other treatments with IFN-α or imatinib. Three patients in molecular relapse after SCT had transient molecular remissions, but two had also been treated with donor lymphocyte infusions. These results, while promising, do not demonstrate convincingly the efficacy of the vaccine. In a subsequent, more stringent, trial using the a similar peptide combination Bocchia *et al*. in Italy studied 16 b3a2 variant CML patients with residual disease stable for at least 6 months, after a minimum of 12 months' treatment with imatinib or 24 months with IFN-α [55]. Patients received six vaccinations and were then assessed for immunological and disease response. Of 10 patients on imatinib, nine had stable cytogenetic disease and one was in cytogenetic remission for a median of 10 months. Five achieved a complete cytogenetic remission with a negative polymerase chain reaction (PCR) for BCR–ABL in three. Of six patients stable for a median of 17 months on IFN-α treatment with 13% median Ph^+ chromosomes in the marrow, five showed reductions in the percentage of Ph^+ chromosomes, with two reaching complete cytogenetic remission. Five of five patients showed specific responses to the peptide with IFN-γ production, 13 of 14 had proliferative responses and delayed-type hypersensitivity to the peptides was seen in 11 patients. These results

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suggest that clinical responses to BCR–ABL peptides can be induced in patients with CML with low levels of stable disease. These rather modest results raise concerns that the method of vaccine administration or the immune status of the treated patients may be suboptimal, or that BCR–ABL does not induce sufficiently powerful cytolytic T cell responses to CML.

Primary granule proteins

Primary granule proteins (PGPs) are a group of serine proteases, or closely related molecules, found in granulocytes and their precursors. They occur in high concentrations in maturing granulocytes and neutrophils – approximately 1 pg enzyme per cell [56–58]. Two PGPs, PR3 and NE, have been studied in detail. Both are overexpressed in myeloid leukaemia blasts and CD34$^+$ leukaemic progenitors [29,56]. They may be important in maintaining a leukaemia phenotype: PR3 anti-sense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukaemia cell line [43,56] and NE-deficient mice are protected from developing acute promyelocytic leukaemia [59]. NE production by CML cells inhibits normal granulopoiesis in culture, suggesting that NE gives CML clonal dominance over normal granulopoiesis [60–62]. In 1995 we chose to study PR3 as a potential source of LSA and using a reverse immunology approach identified PR1 a nine-amino acid HLA-A*0201-restricted peptide which induced myeloid leukaemia-specific CTL responses [30,31]. PR1 is naturally processed and presented on MHC class I molecules from CD34$^+$ CML cells [63], and the degree of cytotoxicity exerted by PR1-specific T cells correlated with the degree of cytoplasmic PR3 expression by the target [30,31,52,64,65]. PR1-specific CD8$^+$ T cells are found at low frequencies (approximately 1/100 000 CD8$^+$ T cells) in healthy individuals, but in leukaemia patients and after SCT these frequencies may rise one or two logs, respectively [52]. High frequencies of PR1-specific CTL are also found in patients with CML responding to IFN-α, and in patients entering molecular remission after SCT as well as patients with AML [52,64,65]. PGP as a group are immunogenic: 40–60% of healthy individuals have CD4$^+$ and CD8$^+$ T cells recognizing NE, PR3 and cathepsin G. These protein-specific T cells can be expanded *in vitro* and are cytotoxic to CML cells [29]. The occurrence of autoreactive T cells to PGP is not well understood. It appears that T cells recognizing PGP escape the normal process of eliminating autoreactive T cells by the thymus. This may explain why PGP are a target of autoimmune attack in Wegener's granuloma and related vasculitides [66–71].

PR-1 vaccine trials

A pilot study nearing completion evaluated PR1 vaccine in patients with refractory or progressing myeloid leukaemias, including many who relapsed after SCT. Patients received a course of 3-weekly PR1 vaccine with montanide adjuvant and granulocyte–macrophage colony-stimulating factor (GM-CSF) [72]. Increased frequencies of PR1-specific CTLs occurred in 22 of 37 patients. Sixteen responders showed clinical improvement including complete remissions in four patients, and a longer event-free survival for patients who had an immune response compared with those who did not. No patients developed cANCA antibodies or vasculitis. These encouraging results have led to the initiation of several new studies with PR1 in less advanced patients.

WT-1

Mice immunized with WT1 peptide or WT1 cDNA reject WT1-expressing tumours, suggesting that WT1 vaccines might be effective as immunotherapy for WT1 positive leukaemia. In man WT1 protein is overexpressed in a wide range of malignancies, including myeloid leukaemias and MDS [34,73–75]. Furthermore, expression increases as disease progresses, making WT1 an attractive vaccine candidate in otherwise untreatable advanced leukaemia [76,77]. A number of HLA-A2- and HLA-24-restricted WT1 epitopes are known and CTLs specific for WT1 peptides and cytotoxic to myeloid leukaemias are generated readily from normal individuals [36,38,78]. Several investigators have reported the occurrence of WT1-specific CTL in patients with cancers, myeloid and lymphoid leukaemias and healthy volunteers [52,65,79–81]. T cells from patients with leukaemia are polyclonal, often recognizing all four HLA-A*0201 immunodominant peptides, whereas healthy controls had lower frequency responses to fewer epitopes, suggesting that myeloid leukaemias naturally elicit T cell responses to WT1 [81]. WT1 IgM and IgG antibodies can also be detected in patients with haematopoietic malignancies [35,82,83]. These observations suggest that WT1 is highly immunogenic in man and an interesting vaccine candidate for haematological malignancies in general.

WT1 vaccine trials

Oka *et al.* reported the outcome of a phase I clinical study of WT1 peptide-based immunotherapy in patients with breast or lung cancer, MDS or AML [84,85]. Patients received an HLA-A24 9-mer WT1 peptide in Montanide adjuvant at 2-week intervals in a dose escalation study. The vaccine was well tolerated and 18 of 26 patients received three or more vaccinations. The only notable side effect was profound leukopenia in two MDS patients reversed by steroid treatment, which concommittently abrogated the WT1 T cell response. Twelve of the 20 evaluable patients had clinical responses, including reductions in blood or marrow leukaemic blasts or tumour size or tumour markers. Increases in WT1-specific CTL frequency correlated with a clinical response.

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Scheibenbogen and colleagues [65] used a WT1-126 peptide vaccine in 16 patients with AML and 1 patient with MDS. Patients received a median of eight vaccinations (range 3–18) [86]. WT1-specific T cell responses were detected in nine of 13 patients by tetramer analysis and eight of 13 by intracellular cytokine staining. Clinical responses were seen in six of 12 patients, with one patient achieving complete remission for 12 months. The patient with MDS had an improvement in neutrophil and platelet counts, two patients had minor responses with transient reductions in leukaemic blasts and two patients achieved temporary disease stabilization. WT1 transcripts as molecular disease markers decreased in five of these six patients. These very promising results indicate that WT1 vaccination can induce functional CTL responses associated with clinical improvement.

Vaccine developments

Improving the applicability and efficacy of peptide vaccines

Peptide vaccines are attractive to use in clinical trials because they are relatively cheap, easily manufactured to clinical grade and easy to administer. However, they have significant limitations. The first is HLA restriction: vaccine use is restricted to patients who have the appropriate HLA type (usually HLA-A2 or A24). At least 60% of the population lack these HLA types and are ineligible for vaccination. Studies with adoptive transfer of immunity to cytomegalovirus have shown the importance of combining $CD4^+$ with $CD8^+$ antigen-specific T cells to achieve sustained immunity [87]. Inclusion of MHC class II binding peptides in a vaccine to elicit $CD4^+$ cells in combination with $CD8^+$ cells would therefore be a worthwhile goal.

Peptide library screening

Peptide library screening offers a way to find new peptide epitopes. Lymphocytes from individuals selected for HLA haplotypes of interest are screened against a 15 mer overlapping peptide library covering the entire protein sequence. Guided by computer-generated peptide–MHC binding predictions, candidate peptides are synthesized and tested for their ability to induce IFN-γ production and promising peptides are further validated as described above. A cocktail of peptides with broad specificity for most common HLA class I and II types can be produced in this way [88,89].

Heteroclitic peptides

Heteroclitic peptides represent a method to enhance peptide vaccine potency. Heteroclitic peptides are synthetic variations of the natural peptide sequence which retain the same HLA binding specificity and avidity but which have enhanced affinity to the T cell receptor (TCR). Several groups are currently exploring heteroclitic WT1 peptides as vaccines [90–93].

Alternatives to peptide vaccines

The use of whole protein rather than defined peptides offers the opportunity to generate leukaemia-specific CTLs against multiple peptide epitopes, binding to a wide range of HLA class I and II molecules, thus eliminating the constraint of HLA restriction and allowing the generation of $CD4^+$ T cells, which could provide help for $CD8^+$ T cell expansion. The identification of PGP-specific $CD4^+$ and $CD8^+$ T cells in healthy donors [29] supports the development of PGP protein vaccines. Protein vaccines, nevertheless, have shortcomings. They are difficult to manufacture and are inefficient inducers of $CD8^+$ CTL responses as they are processed mainly by APCs for MHC class II presentation to $CD4^+$ T cells [94–96]. As an alternative to proteins, DNA vaccines may ultimately represent the most practical approach to inducing T cell responses to a broad range of MHC class I and II epitopes (reviewed in [97]).

Clinical applications of LSA vaccines – the future

While vaccines could conceivably be used to prevent myeloid malignancies, it is unlikely that vaccines alone could eliminate established disease unless combined with other treatments.

Combining vaccines with allogeneic SCT

The finding of increased frequencies of BCR–ABL-, PR1- and WT1-specific CTL after SCT suggests that GVL could be enhanced by post-transplant vaccination. The transplantation of a healthy donor immune system in a leukaemic recipient offers a unique opportunity to boost GVL by also vaccinating the donor. Immediately after SCT, conditions may be favourable for antigen-specific T cell expansion because the preparative regimen creates a lymphopenic environment causing a surge of interleukin (IL)-12 and IL-15 which stimulates lymphoproliferation strongly [98–100]. T cells recently activated by antigen can be boosted favourably by vaccine during this period. However, before vaccination can be applied effectively in SCT recipients it will be necessary to improve methods to selectively prevent acute graft-*versus*-host disease (GVHD) eliminating the need for post-transplant immunosuppression. The combination of the potent GVL effect of the allograft with the vaccine boost for leukaemia-specific T cells could prove to be a highly effective strategy to control refractory leukaemias.

Combining vaccines with non-transplant treatments

Low disease burdens in CML patients stabilized to low levels of molecular disease by imatinib, patients with AML in

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remission or patients with early MDS offer ideal opportunities for vaccination. Chemotherapy, like SCT, can induce a favourable lymphopenic milieu conducive to rapid expansion of vaccine-boosted T cells but may also blunt T cell responsiveness. To optimize vaccine efficiency, it may therefore be necessary to collect antigen-stimulated lymphocytes by apheresis before chemotherapy, re-infusing them with further vaccination following lymphoreductive chemotherapy. Indeed, work published recently by Rapoport and colleagues supports the feasibility of this approach [101]. They performed a randomized Phase I–II trial in 54 patients with advanced myeloma to determine whether combination immunotherapy consisting of vaccination with the pneumococcal conjugate vaccine (PCV) and adoptive T cell transfer could correct the immunodeficiency and lymphopenia induced by high-dose chemotherapy. They demonstrated that individuals who received a single early post-transplant infusion of *in vivo* vaccine-primed and *ex vivo* co-stimulated autologous T cells followed by post-transplant booster immunizations had accelerated immune reconstitution and enhanced antigen-specific $CD4^+$ and $CD8^+$ T cell function *in vivo*.

We are therefore exploring the strategy of inducing lymphopenia (with or without allogeneic transplantation) followed by PR1 and WT1 peptide vaccination to selectively expand leukaemia-specific CTLs during the phase of lymphocyte recovery.

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