**Ph positive-leukemias**

**Introduction**

Ph-positive leukaemias are clonal disorders characterized by the Philadelphia (Ph) chromosome genetic abnormality, which arises from the reciprocal chromosomal translocation t(9;22) (q34;q11). This translocation fuses the genes encoding BCR and ABL, resulting in expression of the constitutively active protein tyrosine kinase, BCR-ABL. Depending on the precise translocation breakpoints and differential mRNA splicing, various molecular weight isoforms of BCR-ABL are generated. These isoforms are associated with distinct types of leukemia. Most (>90%) of patients with chronic myeloid leukaemia (CML) and one third of patients with Ph acute lymphocytic leukaemia (ALL) express the 210-kDa oncoprotein. Twenty percent to 30% of cases of Ph ALL and a few cases of CML are associated with 185-kDa BCR-ABL. A subset of patients with indolent CML expresses the 230-kDa BCR-ABL oncoprotein.

Differences in intrinsic kinase activity and cell context may influence the type of leukemia that arises with each BCR-ABL isoform and the development of therapeutic agents to treat these BCR-ABL–driven malignancies. Although imatinib has emerged as a very powerful drug, most of the patients who achieved responses are at risk of relapse because the leukaemic stem cell pool has not been eradicated. In this paper, we will review some data on the pathophysiology of the disease focusing mainly on stem cells.

**Ph positive stem cells**

In Ph leukaemias a number of experimental studies suggest that a primitive population of cancer stem cells (CSCs) has escaped the normal control of self-renewal resulting in leukaemic development. These CSCs have been implicated in the pathogenesis of relapse and may disclose a number of features of resistance. A critical issue in CML is that despite a rapid decline of the tumour burden with Imatinib, this new agent fails to eliminate residual leukaemic cells, especially leukaemic stem cells. Normal haematopoietic stem cells are rare, generally quiescent but with the capacity of self-renewal and multilineage differentiation. These cells express CD34 but not lineage or activation markers. In CML, haematopoietic stem cells exhibit particular features. CML, is essentially a myeloproliferative disorder which involves a pluripotent stem cell which gives rise to progenitors in permanent cycling state, in the presence of highly
quiescent stem cell population. The molecular mechanisms involved in this deep quiescence are largely unknown but it is thought that this rare, quiescent stem cell population undergoes episodically cell cycle, generating a pool of “active” stem cells responsible of the propagation of the disease. CML stem cells exhibit properties strikingly similar to those of normal haematopoietic stem cells such as self-renewal, pluripotency and quiescence, explaining the difficulties in their isolation and studies. The Ph+ stem-cells are CD34+, CD38-, CD90+, Lin- and the Ph+ translocation can be detected in all the myeloid, erythroid and lymphoid lineages but rarely in T cells.\(^7\) The activation of BCR-ABL in haematopoietic stem cells leads to preferential expansion of myeloid progenitors and differentiated progeny in the blood and bone marrow of the patients. Recent studies also suggest a leukaemia stem cells hierarchy with both short and long term repopulating cells. Of interest, the role of the promyelocytic leukaemia protein (PML) tumour suppressor has been investigated in CML.\(^8\) PML functions as a tumour suppressor controlling processes such as apoptosis, cellular proliferation and senescence. PML expression is high in normal haematopoietic stem cells as well in CML chronic phase cells. However patients with low PML expression would achieve significant higher complete cytogenetic responses and might have a significant better outcome. Thus an induced down-regulation of the expression of PML in haematopoietic stem cells would be useful in clinic. In fact arsenic trioxide, a drug which down regulates PML expression, might disrupt haematopoietic stem cells quiescence and increase the efficacy of anti-leukaemic therapy of drug such as cytarabine.

**Mathematical models in CML**

CML has been one of the most well studied leukaemic disorders. However, questions such as the unique role of the BCR-ABL oncogene, the persistency of leukaemic dormant cells and molecular resistance remains unresolved. More recently the unsatisfactory effect of imatinib on leukaemic stem cells has been a matter of discussion and in vitro studies. Thus several mathematical and computational techniques have been developed to elucidate the mechanisms of CML stem cell persistence.\(^9\)

In 2005, Michor et al. used a differential equations model to explain a biphasic decline of BCR-ABL transcript levels during the first year of treatment with imatinib.\(^10\) The model also explains the early relapse when imatinib therapy is interrupted. In this model, the dynamics of two non-interacting populations of malignant and normal cells is described. More precisely, four layers of the differentiation hierarchy of the haematopoietic system is described. In this model, stem cells give rise to progenitors which produce differentiated cells, which produce terminally differentiated cells. As this hierarchy applies for both normal and leukaemic cells, the leukaemic cells population comprises leukaemic stem cells and 3 different types of leukaemic differentiated cells. Based on the analysis of 169 CML patients, and on a subgroup of responders, the biphasic exponential decline of the leukaemic cells is analyzed. As a result it is assumed that the first slope represents the turnover of differentiated leukaemic cells with a life span of 20 days and the second slope the turnover rate of leukaemic progenitors with a life span of 125 days. Thus imatinib treatment leads to a competitive disadvantage of leukaemic progenitors and leukaemia differentiated cells with a dramatic reduction of their production rates. Also, the rate of increase of BCR-ABL transcript after cessation of the treatment or in case of mutation in the ABL gene is consistent with the presence of leukaemic stem cell. The model concludes that leukaemic stem cells are not affected during imatinib treatment.

In a subsequent paper Roeder et al developed...
a agent-based model of stem cell differentiation. Based on two different datasets, they assumed that BCR-ABL 1 transcript dynamics during imatinib treatment of CML could be explained by a selective functional effect of imatinib on proliferative leukaemic stem cells. In CML, the chromosomal translocation causes an impaired proliferation control together with altered cell-microenvironment interaction. This implies that the malignant clone competes against normal cells. The model subdivides the leukaemic stem cells into 2 compartments: proliferating and quiescent stem cells. Imatinib is assumed to induce the inhibition of proliferative activity and degradation of proliferating stem cells. The analysis was performed on 69 newly diagnosed CML patients from the IRIS trial and 68 patients previously published. Based on the outcome of these 2 set of patients and their responses to the treatment, the model also predicts that a continuous induction of cycling activity in combination with imatinib might produce a better reduction of the leukaemic clone. In addition such strategy would also be useful to reduce the risk of tumour relapse in case of imatinib discontinuation. To summarize, this model predicts the existence of imatinib-sensitive and imatinib-resistant cells population. The issue of drug resistance has been addressed by Komarova and Wodarz. They consider a stochastic dynamical system based on measurable parameters, such as the turnover rates of tumour cells and the rate at which resistant mutants are generated. They postulate that resistant mutant arises mainly before the start of treatment. Data suggest that the 2 main types of mutations which confer resistance to the cells are amplification of BCR-ABL or point mutation in the target protein. Based on the model, they suggested that a combination of 3 targeted drugs with different specificities might overcome the cell resistances. Although these models may explain the reason why patients are at high risk of relapse if imatinib therapy given alone is stopped, they do not take into account the crucial role of the immune system in CML. There are numerous data supporting the role of the immune system in CML. Even during imatinib therapy, recent experiments suggest a robust but transient anti-leukaemia effect of imatinib involving both CD4+ and CD8+ T cells.

A new mathematical model has been recently developed which provide insights into the dynamics and potential impact of the anti-leukaemia effect of the immune response. The model suggests that the balance between immune down-regulation and T cell stimulation by leukaemic cells determines the effectiveness of the anti-leukemia T cell response. In addition, a novel concept of “optimal load zone” is described which correspond to the range of leukaemic cell concentrations were the T cell stimulation rate is faster than the T cell death rate. The model also indicates that vaccination approaches in combination with imatinib could optimally sustain the anti-leukemia T-cell response to potentially eradicate residual leukaemic cell.

Therapeutic approaches based on leukaemic cell proliferation models

Stimulation of quiescent cells

Different approaches have been developed in order to eliminate quiescent leukaemia stem-cells. One hypothesis is that by stimulating these quiescent cells, the rate of entry into cycle could be increased and thereby response to imatinib could be restored. Prior exposure to growth factors such as granulocyte-colony-stimulating factors (G-CSF) of cells form acute myeloid leukaemia patients, enhance their sensitivity to cell cycle specific agent such as cytarabine. In CML G-CSF might stimulate the entry of quiescent CML stem cells into cycle and increase their sensitivity to Imatinib. In order to test this hypothesis,
CD34+ leukaemic cells from newly diagnosed chronic phase CML patients were cultured with or without G-CSF or imatinib either continuously or intermittently. This in vitro assay demonstrated that intermittent but not continuous exposure to G-CSF significantly accelerated the disappearance of quiescent CD34+ CML cells. Based on their in vitro data, a clinical trial has been initiated in order to evaluate the potential clinical use of growth factors in combination with Imatinib. However, this trial stopped prematurely.

In another set of experiments CML and normal CD34+ cells were labeled with 5-(and 6-)carboxyfluorescein diacetate succinimidyl ester (CFSE) to track cell division and cultured in low or high concentrations of growth factors to determine effects of growth factor stimulation on no dividing cells. High growth factor concentrations significantly enhanced CML proliferation with or without imatinib treatment and significantly reduced the number of viable, no dividing CFSE bright cells remaining after imatinib exposure. Stimulation with high growth factors before imatinib treatment further reduced the number of residual no dividing CML CD34+ cells. Importantly, clinically achievable concentrations of granulocyte macrophage colony-stimulating factor alone or in combination with granulocyte colony-stimulating factor also significantly reduced no dividing CML CD34+ cells. These results support the potential efficacy of growth factor stimulation in reducing the residual leukaemia progenitor population in imatinib-treated patients. However, one potential drawback of these approaches could be the stimulation of a quiescent stem cell bearing an ABL-kinase mutation under the threshold of detection. Indeed, it has been well established that ABL-kinase mutations can occur at the level of very primitive stem cells detected by long-term culture assays (Sorel et al., BBRC 2005).

**Immune engineering**

CML has been recognized as a potent model for immune therapy in humans because there is a specific gene rearrangement, BCR/ABL, which product, P210bcr/abl can be the target antigen for immune therapy. Peptides spanning the junction between BCR and ABL in P210bcr/abl are specific to CML cells; they are not present in other normal cells neither in CML patients nor in cells in normal individuals. There are also other potential targets for vaccines in CML including PR1, Wilm tumour protein (WT1), minor histocompatibility antigens, CML-66, CML-28, the ribonucleoprotein telomerase (hTERT) surviving, myeloblastin.10,19,20

A number of studies have focused on different antigens derived from normal tissue proteins than can play a role as tumour antigens in CML. Of these, proteinase 3, a differentiation antigen associated with granule formation in myeloid cells, is aberrantly expressed in tumour cells. PR1, an HLA-A2-restricted peptide derived from proteinase 3, elicits Cytotoxic T lymphocytes that kill myeloid leukaemia cells but not normal marrow cells. They are present at significant frequencies in CML patients in remission. Thus, PR1-specific Cytotoxic T cells may help to eradicate leukemic cells after IFN-α or stem cell transplantation. Two different populations of PR1-specific Cytotoxic T cells have been identified based on their avidity. High-avidity PR1-specific T cells kill CML cells more effectively than low-avidity T cells. Low-avidity PR1-specific T cells have been identified and selectively expanded in vitro from the blood of newly diagnosed CML patients. Circulating high-avidity PR1-specific T cells were identified in patients who achieved cytogenetic responses after therapy IFNα. Thus high-avidity PR1-specific T cells expanded in vitro could be used for therapeutic purposes. The product of the Wilms’tumour gene WT1, a transcription factor expressed at
low levels in immature CD34+ progenitors cells, is over expressed in the leukaemia cells of a large number of CML patients. The HLA-A2 restricted WT1-126 peptide elicits Cytotoxic T cells that specifically kill HLA-A2 leukaemia CD34+ leukaemia cells.

*Ex vivo* responses of CD8 T cells from CML patients to extrajunction bcr-abl peptides and telomerase 540-548 hTert, PR1, and WT1 peptides were recently characterized. CML-specific CD8 T cells were present in most treated patients and were usually multiepitopic: WT1, hTert, PR1, and bcr74 tetramer+ cells were detected in 85, 82, 67, and 61% of patients, respectively. CML-specific tetramer+ CD8 T cells had a predominantly memory phenotype, an intermediate perforin content, and low intracellular IFNγ accumulation in the presence of the relevant peptide. However, in short-term culture with HLA-matched leukemia cells, the patients’ memory T cells were specifically reactivated to become IFNγ-producing effector cells, suggesting that CD8 T cell precursors with lytic potential are present in vivo and can be activated by appropriate stimulation. These cells could be used in patients achieving minimum residual disease after Imatinib therapy.

Ph-positive CML patients express either b2a2 or b3a2 fusion transcripts, depending on whether exon b3 has been included. When translated, b2a2 and b3a2 mRNA each generates a 210-kDa BCR-ABL protein (p210), which is necessary and sufficient for leukemic transformation in experimental models. The BCR-ABL junction in b3a2 mRNA disrupts a triplet codon, producing a novel lysine (K) at the junction in the b3a2 BCR-ABL protein product. A codon disruption also occurs at the b2a2 fusion junction (Asp altered to Glu), but in this case the novel amino acid may not be recognized as it is also present at the normal a1a2 junction. Oligopeptides derived from the BCR-ABL junction are potential novel CML-specific antigens and may therefore elicit an immune response. In addition a number of class I HLA molecules have been reported to bind strongly to peptides spanning the BCR-ABL fusion junction.

The group of Bocchia identified BCR-ABL b3a2 junctional 9-mers that bound strongly to HLA-A3 and A11 (sequence KQSSKALQR) and B8 (sequence GFKQSSKAL). These peptides also elicited specific class I-restricted CTL activity. HLA-A2 is capable of binding a different b3a2 fusion peptide that is able to elicit CTL responses in healthy donors and CML patients. These data suggest that certain BCR-ABL junctional peptides may preferentially bind to certain HLA alleles. However, no sequences from the b2a2 junction bound to any of the HLA class I molecules.

It has also been demonstrated that the HLA-A3 binding peptide from the bcr3abl2 region is endogenously processed and presented on leukaemic cells. Finally, the expression of HLA-B8 and HLA-A3 co-expressed with HLA-B8 reduced the risk of acquiring CML. Taking together, these data suggest that T cell immunity against bcr/abl might possibly be of clinical relevance.

Thus, based on these previous observations, phase I, II trials vaccine trials using a mixture of five b3a2-derived peptides plus the immunological adjuvant QS-2117 have shown peptidespecific T-cell responses, without tumour responses.

The group of Bochia performed more recently a phase-II vaccine multicentre trial in Italy, enrolling patients with b3a2-related CML (b3a2-CML) from four centres. Patients with persistent stable disease during conventional treatment (minimum duration of previous treatment, 12 months (imatinib); 24 (interferon alfa) and with at least one of the following HLA molecules were eligible for the study: HLA A3, A11, B8, DR11, DR1, or DR4. They used a CMLVAX100 vaccine which con-
sisted of 5 b3a2 breakpoint-derived peptides. They enrolled 16 patients with chronic phase CML previously treated with Imatinib or IFNα. They were given six vaccinations with a peptide vaccine derived from the sequence p210-b3a2 plus molgramostim and QS-21 as adjuvants (CMLVAX100); Imatinib (10 patients) and IFNα (6 patients) therapy was continuously administered during vaccine therapy. Patients had cytogenetic improvement including 2 of them in complete molecular response. Of interest 70% of patients had a positive delayed-type hypersensitivity reaction, and in some cases in vitro CD4 proliferative response to b3a2 peptides. Although this phase II trial is of interest, the vaccine strategy dose need specific requirement and a longer follow up of the patients is needed to assess the benefit of antileukemic T-cell mediated immunotherapy.

Interferons (IFN) are cellular glycoproteins with antiproliferative, antiviral and immunoregulatory properties. IFNα exert various effects in the immune system such as modulation of immunoglobulin production, inhibition of T-cell cytotoxicity, monocytes/ macrophage function and natural killer cell activity. The mechanism of action in CML has been extensively studied and IFN has been used in CML patients resulting in long term outcome for those who obtained sustained complete cytogenetic response.31

Interestingly the interferon consensus sequence binding protein (ICSBP) in mice is of interest in order to understand the fundamental role of IFN-α in CML pathogenesis. ICSBP (also known as IFN regulatory factor 8) is a member of the transcriptional factors family called IFN regulatory factors, implicated in the regulation of transcription of IFN-activated genes.32 ICSBP is expressed mainly in haematopoietic cells.33,34 Its expression is strongly induced by IFN-γ and in vivo by IFN-α treatment in CML patients, among whom ICSBP expression is impaired.35,36 The overexpression of BCR-ABL in mice leads to a CML-like disease associated with a down-regulation of ICSBP, whereas the forced co-expression of ICSBP in such mice inhibits the BCR-ABL-induced cell proliferation. Furthermore, ICSBP−/− mice develop a granulocytic leukemia with enlarged lymph nodes, liver and spleen, resembling to CML in human.37 Finally, ICSBP is essential for the generation of plasmacytoid dendritic cells (pDCs, also named IPCs for IFN-producing cells), the major IFN-α producing cells in vivo, since ICSBP−/− mice have a completely depleted pDC compartment, in addition to various phenotypic and functional defects of the other dendritic cell (DC) subsets. Rescuing such mice with retroviral ICSBP transduction in ICSBP−/− progenitors restores a functional pDC population.38,39

Our group recently evaluated the number of pDCs and their function in CML patients either in CP or in complete remission after IFN-α or IM therapy. Our results demonstrate a functional inability of PBMCs to produce IFN-α in CML patients, associated with a massive depletion of the pDC compartment. This impairment, which is a direct consequence of p210BCR-ABL activity, underlines a central role for the homeostasis of both pDCs and IFN-α production in the pathogenesis of CML.

Finally, and more recently, it has been demonstrated that IFN-α promotes the proliferation of dormant haematopoietic stem cells.40 IFN-α treatment results in the phosphorylation of STAT1 and PKB/Akt, expression of IFN-α target genes in stem cells, as well as stem cell antigens (Sca-1) upregulation at their surface. Thus this activation of “dormant” stem cells could be one of the mecanism of action of IFN-α in CML.
Conclusions

The treatment of CML patients has been recently revolutionized by Imatinib. The rate of molecular responses is high and the survival of the patients has improved significantly. However, increasing evidence suggests that some CML stem cells are resistant to Imatinib. In addition there are some concerns for the use of Imatinib for a long period of time because of the risk of unexpected late side effects and for economic reasons. Thus, novel therapeutic strategies are needed to target efficiently leukemic stem cells. Beside the development of novel anti-TK drugs, several current strategies currently include the combination therapies using TKI, growth factors, interferon or arsenic trioxide.

References


28. Pinilla-Ibarz J, Cathcart K, Koronskit T, Soignet S,


