Survivin isoform expression patterns in CML patients correlate with resistance to imatinib and progression, but do not trigger cytolytic responses

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Abstract Tyrosine-kinase inhibitors are very effective in patients with CML, but in most cases the disease relapses after their discontinuation. As a result, novel approaches should be considered, such as anti-survivin treatment or anti-survivin-based immunotherapy. To gain insight into the roles of survivin isoform expression and specific CD8+ T cells in CML, we investigated 51 patients at different stages, both at diagnosis and during treatment. We demonstrated that (i) patients at advanced-stage displayed an increased expression of the standard-survivin form along with a significant decrease of survivin-2B and -ΔEx3 levels, (ii) patients in chronic phase with higher expression of the standard-survivin exhibited a 3.5-fold increased probability not to achieve an optimal response to imatinib (p = 0.048), (iii) responders displayed a significant up-regulation of all survivin isoforms in bone marrow, and (iv) anti-survivin CD8+ T cells were undetectable both at diagnosis and during treatment. Accordingly, our results question the validity of immunotherapeutic approaches targeting survivin in CML.

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1. Introduction

The treatment of patients with chronic myeloid leukemia (CML) has dramatically been changed over the last years, following the admission of tyrosine-kinase inhibitors (TKIs). Although the majority of patients treated with TKIs display a complete cytogenetic response (CCyR) and/or a major molecular response (MMR), their discontinuation results in rapid reappearance of the disease, and consequently patients need to receive their treatment for longer time periods [1,2]. As a result, in recent years, there is an increased interest in also treating such patients with other modalities and in particular utilizing vaccination approaches with tumor peptides, which could expand anti-CML-specific T cell clones that could eradicate residual disease, leading to possible cure [3,4]. To this end, it is noteworthy that even before the treatment of CML with TKIs, the disease was curable only through immune-mediated approaches, such as the elimination of leukemia cells by allogeneic stem cell transplantation or donor T-lymphocyte infusion (DTI) [3,4]. One of the proteins proposed for CML immunotherapy is survivin, since it is expressed by CML malignant cells [5–7], and consequently the expansion of anti-survivin CD8+ T cells could be an alternative therapeutic approach for the treatment and possibly cure of such patients [8].

Survivin is a member of the inhibitors-of-apoptosis (IAP) family of proteins that is involved in the control of cell division, cytokinesis and the cell death regulation [9,10]. Survivin is present during fetal development but is undetectable in terminally differentiated adult tissues. However, the survivin gene is reactivated in nearly all human cancers and hematologic malignancies and the majority of them express the protein at high levels [5,9,11,12]. Considering CML, recent studies have reported that the expression of survivin is regulated through BCR-ABL/MAPK signaling, at both transcriptional and posttranslational levels [13,14], and it was higher in the accelerated phase/blast crisis (AP/BP) than in the chronic phase (CP) of the disease [5,7].

Recently, several reports have demonstrated that alternative splicing of the survivin gene can give rise to five different mRNA isoforms, namely standard survivin (survivin-std), survivin-2B, survivin-ΔEx3, survivin-3B and survivin-2a [15–18]. Survivin isoforms have unique subcellular localization patterns that could be associated with unique functional properties, while they vary in their effect on apoptosis [15]. Thus, while survivin-std and ΔEx3 retained an anti-apoptotic function [15], survivin-2B and -2a had a proapoptotic function, acting thus as natural antagonists against survivin-std [17,19]. On the other hand, the localization and the role of survivin-3B in apoptosis remain unknown.

The presence and the role of survivin isoforms in CML biology and prognosis are thus far unclear. This study was scheduled in order to examine the expression of survivin isoforms, as well as the presence of anti-survivin-specific CDB T cell responses in CML patients, at diagnosis and during imatinib treatment. We identified a specific pattern of survivin isoforms expression according to the disease stage, the type of bcr-abl rearrangement and the result of treatment, with a higher expression during the recapitulation of bone marrow by non-malignant cells. We further demonstrated that anti-survivin CDB T cell responses were undetectable both at diagnosis and remission. Accordingly, these results suggest that anti-survivin therapeutic approaches must be taken with caution in patients with CML.

2. Materials and methods

2.1. Patients

Fifty-one Ph+ CML patients (male/female: 26/25, mean age: 58.5 years, range: 22–78) who had been diagnosed in our institutions between December 2000 and September 2009 entered this study. At enrollment, all patients displayed the Philadelphia chromosome [(t(9;22)(q34;q11)) and one (CML in chronic phase), an additional chromosomal defect (monosomy 7). Among them, 43 were analyzed during chronic phase (CP); 37 at diagnosis and 6 when receiving interferon-alfa (IFN-a) (3) or hydroxyurea (3) without any cytogenetic response. Eight patients were analyzed during the accelerated phase (AP) or the blast crisis (BC) of the disease; in particular, the patient with monosomy 7 progressed in BC 6 months after enrollment, one patient was diagnosed at BC (bone marrow blast 40%), one in AP, while 5 others were followed in other centers, receiving IFN-a (1), IFN-a plus aracytin (1), hydroxyurea (2) or imatinib (1) and their genetic material was available only during BC (2) or AP (3). Nineteen patients carried the b3a2-BCR-ABL rearrangement (37.2%); 23, the b2a2-BCR-ABL (45.1%); 7, both b3a2 and b2a2 (13.7%); 1, the e1a2 (the patient who displayed BC during imatinib treatment); and 1, the rare c3a2 (e19a2) rearrangement. Clinical and laboratory characteristics of the patients are presented in Table 1. All patients received imatinib mesylate (Glivec, Novartis, Switzerland), except one in BC who received nilotinib plus chemotherapy. Moreover, the patient with monosomy 7 was receiving IFN-a at enrollment and changed to imatinib only after the disease progression to BC. Serial samples from peripheral blood (PB) and bone marrow (BM) were selected for the great majority of them, with the exception of 3 patients in CP, since after the achievement of hematologic response, they were followed in other centers.

At the end, PB and BM (performed during follow-up) from 7 patients with non-Hodgkin lymphoma (NHL) in complete remission and far off any therapy and PB from 8 normal individuals were used as controls.

The study was conducted in accordance with the principles of Helsinki declaration and was approved by the Institutional Review Board. All subjects gave informed consent and the procedures followed were in accordance to institutional guidelines.

2.2. Molecular analyses

Total RNA was isolated from PB and BM aspirates using TRI (Ambion, Austin, USA), according to manufacturer's instructions. cDNA was reversed transcribed from 1 mg of the RNA, using a random 6-mer oligonucleotide primer (50 pmol/mL) (Roche Diagnostics, Basel, Switzerland) and M-MLV reverse transcriptase (Invitrogen, Paisley, UK), according to manufacturer's instructions. Subsequently, the disease burden was estimated using quantitative-real time reverse transcription-polymerase chain reaction (Q-RT-PCR) and FusionQuant standards (Ipsogen, Marseille, France) according to a
Survivin isoform expression patterns in CML patients

Table 1  Characteristics of the patients with CML.

<table>
<thead>
<tr>
<th>Patients (no.)</th>
<th>Chronic phase</th>
<th>Accelerated phase/blast crisis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>At diagnosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Under treatment a</td>
<td></td>
</tr>
<tr>
<td>Age (median, range)</td>
<td>57.4 (22–78)</td>
<td>62.2 (43–72)</td>
<td>61.6 (34–78)</td>
</tr>
<tr>
<td>Sex (Male/female)</td>
<td>22/15</td>
<td>2/4</td>
<td>2/6</td>
</tr>
<tr>
<td>WBC (x10^9 L^-1) (mean, SDEV)</td>
<td>90.7±74.6</td>
<td>40.9±59.1</td>
<td>134.2±166.8</td>
</tr>
<tr>
<td>Hb (g/dl) (mean, SDEV)</td>
<td>12.4±1.4</td>
<td>11.4±1.3</td>
<td>10.3±2.1</td>
</tr>
<tr>
<td>PLT (x10^9 L^-1) (mean, SDEV)</td>
<td>622.6±592.7</td>
<td>244.3±98.4</td>
<td>371.0±355.3</td>
</tr>
<tr>
<td>Basophils (x10^9 L^-1) (mean, SDEV)</td>
<td>3.0±8.0</td>
<td>0.5±0.7</td>
<td>7.3±16.2</td>
</tr>
<tr>
<td>PB blasts (%) (mean, SDEV)</td>
<td>0.7±1.4</td>
<td>0.1±0.4</td>
<td>14.3±11.2</td>
</tr>
<tr>
<td>BM blasts (%) (mean, range)</td>
<td>2.1±2.1</td>
<td>2.1±2.4</td>
<td>21.7±12.0</td>
</tr>
<tr>
<td>bcr-abl subtype (b3a2/b2a2/both)</td>
<td>14/18/4 b</td>
<td>2/3/1</td>
<td>3/2/2 c</td>
</tr>
<tr>
<td>Disease burden (%) (mean, range)</td>
<td>133.9 (36.3–444.1)</td>
<td>80.8 (10.3–178.3)</td>
<td>118.2 (39.0–307.8)</td>
</tr>
<tr>
<td>Spleen (%)</td>
<td>51.3</td>
<td>83.3</td>
<td>100</td>
</tr>
<tr>
<td>Response to imatinib (CCyR/MMR) (%) d</td>
<td>72.9</td>
<td>33.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Follow-up (mean, range) d</td>
<td>41.8 (6–97)</td>
<td>55 (6–96)</td>
<td>26.6 (1–96)</td>
</tr>
</tbody>
</table>

Abbreviations: PB, peripheral blood; BM, bone marrow; CCyR, complete cytogenetic response; MMR, major molecular response; SDEV, standard deviation

a Three patients were receiving interferon-α and 3 hydroxyurea.
b A patient carried the e19a2 (c3a2)/bcr-abl rearrangement.
c A patient carried the e1a2/bcr-abl rearrangement.
d The patients followed-up in other centers after diagnosis were excluded from this analysis.

standardized protocol [20]. Survivin-std, survivin-2A and survivin-3B mRNA expression was detected by reverse-transcriptase PCR (RT-PCR), while survivin-2B and survivin-ΔEx3, by quantitative real-time reverse-transcriptase PCR (Q-RT-PCR), as described in detail by us in a recent publication [21]. The expression of the analyzed genes in CML and control group samples was calculated as a multiple of the gene expression in a normal bone marrow material obtained from a patient with NHL in complete remission for 5 years, which served as calibrator. Subsequently, the relative quantification and the calculation of the range of confidence were performed using the comparative ΔΔCT method, as described [22].

Genomic DNA was extracted from BM aspirates or PB using the QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK), and HLA-typing was performed by PCR-SSP (Protrans SSP kits, Protrans, Germany), according to manufacturer’s instructions.

2.3. Western blot analysis

The survivin protein expression was determined in randomly selected samples from CML patients (before and after treatment) and controls, using an anti-survivin polyclonal goat antibody (ab27468) from Abcam (Cambridge, UK), according to a previously described protocol [23]. GAPDH (rabbit polyclonal, code: 2275-PC-100; Trevigen, Gaithersburg, MD, USA) served as a loading control.

2.4. Peptides, class I HLA-multimers and frequency of survivin-specific precursor CTLs

Five CML patients expressing HLA-A2 and/or −A24 were analyzed for the presence of specific anti-survivin precursor CTL (pCTL) responses, both in PB and BM. Measurement of pCTLs, specific for two naturally processed and presented peptides of survivin (LTLGEFLKL presented by HLA-A2) and survivin-2B (AYACNTSTL presented by HLA-A24), was performed in these patients at diagnosis and was repeated 6–12 months after imatinib treatment. These peptides were used on the basis of their ability to induce spontaneous CTL responses in cancer patients, and because they are the only ones used in immunotherapeutic trials [24–26]. As controls, peptides of melanoma-associated antigen family A1 (MAGE-A1), MAGE-A3, and human telomerase reverse transcriptase (hTERT) were used. The peptides used according to the HLA phenotype of the patient were EADPTGHSY (MAGE-A1.A1 presented by HLA-A1), EADPTGHSY (MAGE-A1.B35 presented by HLA-B35), EVDPIGHLY (MAGE-A3.A1 presented by HLA-A1), FLWGPRAVL (MAGE-A3.A2 presented by HLA-A2), TFPDLESEF (MAGE-A3.A24 presented by HLA-A24), EVDPIGHLY (MAGE-A3.B35 presented by HLA-B35), ILAKFLHWL (hTERT-ILA.A2 presented by HLA-A2), RLFYRKSV (hTERT-RLF.A2 presented by HLA-A2) and VYAETKHFL (hTERT.A24 presented by HLA-A24).

All peptides were synthesized on solid phase using F-moc for transient NH2-terminal protection, purchased as lyophilized at >90% purity ascertained by mass spectrometry (Abgent, San Diego, USA), dissolved in DMSO at 10 mg/mL and stored at −20 °C before use. Class I HLA-multimers containing all peptides labeled with phycoerythrin (PE) as well as control multimers containing EBV and influenza peptides and labeled with allophycocyanin (APC) were prepared.

Pepptide-specific pCTLs were detected in PB and BM using a sensitive methodological approach combining HLA-multimer flow cytometric technology with a previous step of in vitro amplification with multiple peptides under limiting dilution conditions. Based on the number of pCTLs detected in each sample, their frequencies in the PB or BM were estimated by
the use of Poisson distribution analysis for rare events. Detailed description of this methodology has been recently presented in our previous publication [27].

2.5. Statistical analysis

Mann–Whitney U test, logistic regression and Spearman’s correlation analyses were appropriately performed by the use of SPSS statistical software (version 10.0, Chicago, IL, USA).

3. Results

3.1. Response of CML patients to imatinib treatment

Twenty-nine out of 40 CML patients in CP (65%) who followed-up in our centers achieved a sustained CCyR and/or MMR under 300–400 mg of imatinib, with a median follow-up of 57.3 months (range: 7–96 months); two of them (5%) exhibited also a complete molecular remission (CMR). Four of the above patients (despite the achievement of CCyR/MMR) as well as three others, early after imatinib administration (in total 7 out of 40 CML patients in CP, 17.5%), exhibited grade III/IV toxicity with imatinib and changed their treatment to nilotinib (6) or dasatinib (1); six of them achieved or retained in CCyR/MMR and one achieved a CMR. Moreover, six other patients (15%) did not achieve an optimal cytogenetic or molecular response under imatinib, according to European Leukemia Net recommendations [28] and were receiving either higher doses of imatinib (2), or changed to nilotinib (2) or dasatinib (2); three of them subsequently achieved CCyR and/or MMR. Two out of 4 patients in BC displayed hematologic remission (with 800 mg imatinib and nilotinib plus chemotherapy, respectively) with a slight reduction of disease burden, but without any cytogenetic response. One patient in AP was receiving high doses of imatinib (600 mg/day) and achieved sustained complete cytogenetic and major molecular responses (CCyR, MMR). Finally, 3 patients in BC and 2 in AP died due to disease progression and complications.

3.2. Survivin isoform expression according to different stages of CML

Examples of the RT-PCR expression of survivin isoforms and Western blot analyses are presented in Fig. 1. In the control group, the expression of survivin isoforms was either very low or undetectable in PB, while all isoforms were expressed to a significantly higher level in BM (Table 2). On the other hand, no difference between PB and BM was observed in CML patients at diagnosis. Furthermore, no significant differences were observed between the expression levels of survivin isoforms in CML patients at diagnosis (CP and AP/BC) and the BM of normal control group (Table 2). However, CML patients in AP/BC phase displayed significantly lower levels of Survivin-2B and -ΔEx3 compared to CML in CP (p=0.03 and p=0.011, respectively). Finally, although the expression of survivin-std was higher in AP/BC compared to CP (Table 2), this difference was not significant (p=0.111).

![Figure 1](image-url)
1. Diagnosis (PB) A02, B35  4.0  285  0  <2.5  0  <2.5  2  5.0  0  <2.5  2  5.0  
   (BM)     0.3  35  0  <3.0  0  <3.0  0  <3.0  0  <3.0  1  30  
6-month treatment (PB)  4.8  200  0  <2  0  <2  1  2.0  0  <2  0  <2  
   (BM)     0.6  20  0  <17  0  <17  0  <17  0  <17  0  <17  
2. Diagnosis (PB) A02, A24  2.1  210  0  <4.7  0  <4.7  0  <4.7  0  <4.7  0  <4.7  
   (BM)     2.0  150  0  <5.3  0  <5.3  0  <5.3  0  <5.3  0  <5.3  
6-month treatment (PB)  1.3  53  0  <7.2  0  <7.2  0  <7.2  0  <7.2  0  <7.2  
   (BM)     0.3  10  0  <53  0  <53  0  <53  0  <53  0  <53  
12-month treatment (BM)  0.2  10  0  <119  0  <119  0  <119  0  <119  0  <119  
3. Diagnosis (PB) A24  5.0  282  0  <1.8  0  <1.8  0  <1.8  0  <1.8  0  <1.8  
   (BM)     0.7  35  0  <14  0  <14  0  <14  0  <14  0  <14  
6-month treatment (PB)  2.2  256  0  <4.4  0  <4.4  0  <4.4  0  <4.4  0  <4.4  
   (BM)     0.3  28  0  <120  0  <120  0  <120  0  <120  0  <120  
4. Diagnosis (PB) A02, B35  0.75  20  0  <13  0  <13  0  <13  0  <13  0  <13  
   (BM)     0.3  10  0  <102  0  <102  0  <102  0  <102  0  <102  
6-month treatment (BM)  0.2  7  0  <50  0  <50  0  <50  0  <50  0  <50  
12-month treatment (PM)  2.0  192  0  <5.8  0  <5.8  0  <5.8  0  <5.8  0  <5.8  
   (BM)     1.1  96  0  <8.1  0  <8.1  0  <8.1  0  <8.1  0  <8.1  
5. 12-month treatment (A02)  15  96  0  <8.5  0  <8.5  0  <8.5  0  <8.5  0  <8.5  

Abbreviations: PB, peripheral blood; BM, bone marrow; MLPCs, mixed lymphocyte-peptide cultures; f: frequency of pCTLs specific for the given peptide per 10⁷ peripheral blood CD8⁺; +: positive; see text for the explanation of “+” designation of frequencies. 

a Patients nos. 1, 2, 4 and 5 displayed CCyR/MMR after imatinib treatment, while patient no. 3 exhibited a suboptimal response (no CCyR, reduction of disease burden estimated by Q-RT-PCR only one log.) and changed his therapy to dasatinib.
is noteworthy that the patient with the rare c3a2 (e19a2) rearrangement displayed very low levels of all survivin isoforms at diagnosis, and a molecular remission (established by conventional nested PCR) early after imatinib treatment.

3.3. Expression of survivin and its isoforms after imatinib treatment

In order to determine whether imatinib treatment affected the expression levels of survivin isoforms, these were examined in PB and BM of CML patients in CCyR and/or MMR. Interestingly, the levels of survivin-std were not only significantly higher compared to normal BM, but also compared to its expression in CP ($p < 0.001$), and in AP/BC, although in the latter, the difference was not significant ($p = 0.111$) (Table 3). Moreover, the expression levels of survivin-2B and -ΔEx3 were significantly higher in CCyR/MMR compared to their expression in AP/BC ($p = 0.03$ and $0.01$, respectively) and the expression of survivin-ΔEx3 in CCyR/MMR compared to its expression in CP ($p = 0.006$). However, the expression of survivin isoforms in PB of CML patients in CCyR/MMR was significantly lower compared to their expression at diagnosis, almost similar of their expression in PB of control group (Table 2).

3.4. Detection of anti-survivin pCTLs in PBMCs and BMMCs in CML patients

Prior to assessing for the presence of peptide-specific pCTLs in the microcultures, the multimers were validated for their specificity and sensitivity as described previously [28], in order to detect only peptide-specific populations in MLPCs containing pCTLs specific for the corresponding survivin, survivin-2B, MAGE-A1, MAGE-A3 and hTERT peptides and not control ones (EBV-peptides) (Fig. 2). To estimate the frequency of survivin-specific pCTLs, MLPCs were performed on PB and BM of 5 CML patients, included in the study, at diagnosis and 6–12 months after imatinib treatment (Table 3). Among them, four patients (nos. 1, 2, 4 and 5 of Table 3) displayed CCyR/MMR after imatinib treatment, while one patient (no. 3 of Table 3) exhibited a suboptimal response (no CCyR, reduction of disease burden estimated by Q-RT-PCR only one log.) and changed his therapy to dasatinib.

![Figure 2](image-url)
Table 3 presents, for each patient, the number of CD8+ cells tested, the number of MLPCs performed, the number of MLPCs containing a multimer positive population and the estimated frequency of peptide-specific CTLs. Frequencies designated as "less than" (<) are the minimum frequencies determined on the basis of the maximum number of CD8+ cells obtained from the patients, and among which, one specific pCTL could not be found. This designation was used to declare that the presence of specific pCTls at lower frequencies cannot be excluded.

On the basis of the results of the molecular analysis presented above, whereby a significant increase in the expression of survivin isoforms was observed, the possible expansion of such pCTls after treatment was examined. pCTls against the corresponding survivin or survivin-2B peptides were not detected in PB or BM of all CML patients, both before and after imatinib treatment (Table 3). To exclude whether not detecting survivin-specific pCTls was due to the culture conditions employed, an amplification of pCTls against hTERT, MAGE-A1 and MAGE-A3, under the same conditions, was also performed. Interestingly, pCTls against hTERT and MAGE-A3 peptides were recognized and amplified in a CML patient both in PB and BM at diagnosis, as well as in PB after imatinib treatment (Table 3, Fig. 2). Peptide-specific pCTls against all the above peptides have also been detected in normal individuals and presented in our recent publication [27].

4. Discussion

In this study, we analyzed both survivin isoform expression and the possible expansion of anti-survivin-specific CTL responses in CML patients before and after imatinib treatment. Until now, the majority of published reports on survivin focus on the standard full-length molecule and have not assessed the function of the individual survivin splice variants in various cancer types and in CML in particular [5–7,29]. However, this is an important limitation considering that survivin isoforms exhibit similar and opposing functions. Moreover, regarding survivin-std, there are conflicting results in the literature concerning its expression. This fact may relate to varying specificity of the molecular techniques applied, or the presence of different approaches for storing and processing the tissues. For example, in the study of Shinozawa et al., RT-PCR analysis revealed survivin-std expression in CML patients that was undetectable by Northern blot analysis [5]. In our study, we utilized very sensitive RT-PCR and Q-RT-PCR techniques and our results were also verified, for at least survivin-std, at the protein level. Importantly, we demonstrated that CML patients express survivin isoforms with a different pattern according to the disease stage. In particular, at advanced CML stages (AP/BC) increased survivin-std was observed alongside a significant decrease of survivin-2B and ΔEx3 levels. This pattern could partially explain the biology of the disease progression, especially the low expression of the pro-apoptotic survivin-2B, and might be used as an indicator of the disease status in ambiguous circumstances. Interestingly, this finding is in accordance with other studies, where survivin-2B expression, unlike survivin-std, was inversely associated with disease progression in other malignancies [30–32].

Furthermore, we demonstrated that CML patients at diagnosis with a high survivin-std expression displayed a 3.5-fold increased probability not to achieve an optimal response to imatinib. Until now, survivin expression has been correlated with the response to taxol/platinum-based regimens in ovarian cancer [33], or to endocrine therapy in breast cancer [34], while its inhibition in mice rendered lymphoma tumors more susceptible to immunotherapy [35]. To the best of our knowledge, our study is the first indicating that survivin-std levels correlate with response to targeted therapy. Furthermore, we found that the expression of the pro-apoptotic survivin-2a, correlated significantly with BCR-ABL subtype, whereas patients with b2a2 subtype displayed inexplicably higher levels of survivin-2a compared to patients carrying the b3a2. This finding might explain why patients with b2a2 subtype displayed a better response rate and prognosis after TKI treatment, as reported recently by Lucas et al. [36]. Moreover, patients with the b2a2 subtype also displayed significantly higher levels of survivin-3B; however, both the localization and the function of this isoform are still unknown.

Although survivin-std has a clear anti-apoptotic effect, it is important to point out that the different survivin isoforms may vary in their effect on apoptosis [15,17,19]. Interestingly, we observed that CML patients in AP/BC phase displayed significantly lower levels of the anti-apoptotic survivin-2B and the pro-apoptotic survivin-ΔEx3 compared to CML in CP, and this difference, compared to normal controls, was significant only for the survivin-ΔEx3 isoform. However, the significant decrease of survivin-ΔEx3 isoform might be compensated by the increase of survivin-std expression, which mainly displays an antian apoptotic function.

Interestingly, we found that the expression of survivin isoforms was increased after imatinib treatment in CML patients in CCyR and/or MMR. To this end, it must be noted that while survivin-std is highly upregulated in malignancy vis-à-vis normal tissues, increased survivin-std expression has been found in a number of different types of non-malignant proliferating cells such as colonic crypt cells, hematopoietic progenitor cells, T cells, vascular endothelial cells, testis, thymus, placenta, and many others [9,37–41], and may regulate their proliferation or survival. Thus, it would be plausible to consider that the overexpression of survivin isoforms observed in CML patients after imatinib treatment corresponds to the recapitulating bone marrow by normal (progenitor, vascular endothelial, etc.) cells. As a result, the proposed targeted anti-survivin therapies (reviewed by Fukuda and Pelus [42]) should be considered, at least for CML patients, with skepticism.

Our finding that anti-survivin-std or anti-survivin-2B pCTls were not detected in CML patients, despite the use of a very sensitive methodology, confirms our previous results indicating that anti-survivin immune responses are under a strict immune regulation [27,43]. Indeed, detection of anti-survivin pCTls was not achieved neither in the patient exhibiting responses against other TAAs, nor in patients who presented with elevated expression of the protein at diagnosis and after their treatment. We postulate that suppression of anti-survivin immune responses represents a protective mechanism that reassures the survival of rapidly
regenerating normal hematopoietic, vascular endothelial, and other cells. Moreover, this fact indicates that survivin plays a central role in the protection of these cells against apoptosis. Finally, bearing in mind that the success of immunotherapy depends on the magnitude of the pre-existing immune responses [44,45], our results might add a further piece of evidence against the use of survivin-targeted immunotherapy in CML patients.

5. Conclusion

In this study we observed a distinct pattern of survivin isoform expression in CML patients according to the disease stage, while the antiapoptotic survivin-std expression at diagnosis was associated with the response to imatinib treatment. However, an overexpression of all survivin isoforms was observed in BM of CML patients in CCGR and/or MMR. These results, along with the inability to augment anti-survivin CD8+ T cells, both at diagnosis and during imatinib treatment, question whether the recent interest in the therapeutic targeting of survivin in CML can have any benefit.

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