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FOCUSSED RESEARCH REVIEW

HLA and melanoma: multiple alterations in HLA class I and II expression in human melanoma cell lines from ESTDAB cell bank

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Abstract Altered HLA class I and class II cell surface expression has been reported in many types of malignancy and represents one of the major mechanism by which tumour cells escape from T lymphocytes. In this report, we review the results obtained from the study of constitutive and IFN-gamma-induced expression of HLA class I and II molecules in 91 human melanoma cell lines from the European Searchable Tumour Cell Line Database, and compare them with published data on HLA expression in other types of cancer. Various types of alterations in HLA class I cell surface expression were found in a high percentage (67%) of the studied cell lines. These alterations range from total to selective HLA class I loss and are associated with β2-microglobulin gene mutations, transcriptional downregulation of HLA class I genes and antigen processing machinery components, or with the loss of heterozygosity in chromosome 6. The most frequently observed phenotype is selective downregulation of HLA-B locus, reversible after treatment with IFN-gamma. The expression of constitutive- or IFN-gamma induced-surface expression of at least one HLA class II locus is positive in 71.5% of the analysed cell lines. Four different HLA class II expression phenotypes were defined, and a positive correlation between the expression of class I and II molecules is discussed. More detailed information on the HLA expression patterns and others immunological characteristics of these melanoma cell lines can be found on the following website http://www.ebi.ac.uk/ipd/estdab.

Keywords HLA class I and II · Melanoma · ESTDAB · Tumour

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Introduction

HLA molecules are important mediators of tumour cell recognition by the immune system, mainly by T lymphocytes [20]. Tumour-associated antigens (TAA) expressed by neoplastic cells can be recognized by CD8+ and CD4+ T cells. HLA class I antigens play a key role in immune reactivity against malignant cells because of their ability to bind peptides of TAA for subsequent presentation to CD8 +cytotoxic T lymphocytes [39, 40]. Altered HLA class I expression on malignant cells provides a mechanism of immune escape from CD8+ T cell recognition [12, 13, 15, 33] and frequently correlates with poor survival, disease progression and limited response to immunotherapy [3, 8, 18, 33, 36, 38, 53, 56]. On the other hand, CD4+ T cells recognize complexes of MHC class II molecules with peptides generated from degradation of exogenous or endogenous proteins [25]. The constitutive expression of MHC class II molecules is restricted to the cells of the immune system and thymic epithelial cells [27]. Tumour cells that

express de novo class II antigens are able to present tumour peptides directly to CD4+ T cells, consequently increasing the effectiveness of anti-tumour reactivity. Peptides from many TAAs form complexes with MHC class II molecules for recognition by CD4+ T cells [21, 40, 50]. Therefore, characterization of the tumour HLA class I and II expression is important both for a better understanding of the mechanisms of the anti-tumour immune reactivity and for optimization of the current protocols of cancer immunotherapy.

The altered HLA class I tumour phenotypes can be classified into several major groups depending on the type of HLA loss or downregulation [11]. The different phenotypes of HLA class I antigen surface expression are: Phenotype Ia, HLA class I total loss (structural defects); or Phenotype Ib, HLA class I total downregulation (regulatory defects, reversible with cytokines); Phenotype II, HLA haplotype loss; Phenotype III, HLA-A or -B locus loss or downregulation; Phenotype IV, HLA allelic losses; Phenotype V, compound phenotype; Phenotype VI, unresponsiveness to interferon; Phenotype VII, expression of HLA-E in HLA class I deficient tumours. In addition, it is important to know the nature of the HLA alterations, whether it represents a permanent alteration (Phenotype Ia, II, IV and VI and some cases Phenotype V), or a defects that, in most cases, can be corrected with cytokines or other agents (Phenotpe Ib, III, V). Altered HLA class I phenotypes have been described in primary tumours of different histological origin with different frequency: in 88.5% of breast carcinomas [7], in 90% of cervical cancer [22], in 77% of laryngeal tumours [28], in 74% of colorectal tumours [29], in 90.2% of prostate carcinoma (unpublished observations), and in 77% of bladder tumours [30]. In general, metastatic lesions are characterized with higher incidence of HLA class I losses than primary tumours. For instance, in oesophageal squamous cell carcinoma, class I abnormalities have been reported in 43% of primary tumours and in 90% of metastatic lesions [38]. In melanoma lesions, these numbers range from 48.5% in primary tumours to 81.8% in metastatic lesions [19]. The de novo HLA class II expression has been reported in various types of solid tumours, including melanoma, renal carcinoma and colorectal carcinoma [2, 9, 17, 34].

The objective of this review is to summarize the constitutive and IFN-γ-induced cell surface expression of HLA class I and class II antigens in a large panel of human melanoma cell lines obtained from the European Searchable Tumour Cell line Database (ESTDAB Project contract no. QLRI-CT-2001-01325) [42]. This project represents a part of a collaborative effort to make available a bank of well-characterized human melanoma cell lines (ESTDAB) for the international scientific community.

HLA class I expression in ESTDAB melanoma cell lines

HLA class I surface expression was measured in 91 human melanoma cell lines by indirect immunofluorescence and flow cytometry using a panel of specific monoclonal antibodies against HLA molecules defined earlier [14]. The HLA genotyping and microsatellite analysis of the chromosome 6 were performed for each cell line as described in a previous publication [43]. We have previously reported multiple alterations in HLA class I cell surface expression on 67% of the studied 91 melanoma cell lines (Fig. 1) [37]. The most frequently observed phenotype was downregulation of HLA-B locus, representing Phenotype III. It was observed in 32/91 cell lines (35%) and was reversible after treatment with IFN-γ. In contrast, none of the studied cell lines had downregulation of HLA-A locus. Total HLA class I loss or marked downregulation of HLA class I expression (Phenotype Ia and Ib) was found in 10/91 (11%) cell lines. Out of them two cell lines, 2%, (ESTDAB-038 and ESTDAB-109) showed a total irreversible HLA class I loss (Phenotype Ia) due to β2-m gene mutations, and eight cell lines had total HLA class I downregulation that was recovered after treatment with IFN-γ (9%). Phenotype II (HLA haplotype loss) was found in 13% of the cases. The compound Phenotype V (a combination of LOH in chromosome 6 and downregulation of one or more HLA class I alleles) was found in 6% of the cell lines, while HLA Phenotype VI with resistance to IFN-γ was found only in two cell lines (2% of the cases). In 33% of the studied cell lines we did not find any HLA class I alterations (Fig. 1) [37]. It is important to emphasize that HLA allelic losses could have been overlooked in many of the cell lines due to the lack of allele specific antibodies. Perhaps, this explains why we did not encounter any cell line with Phenotype IV and suggests a possibility that cell lines with HLA allelic losses might be in the group with "no alterations detected". Expression of both constitutive and IFN-induced HLA-E and HLA-G was analysed in 30 melanoma cell lines and in none of the cases we were able to detect a surface expression of these molecules, suggesting that its expression is not a frequent event in melanoma cells [32, 44]. On the other hand, a recent report has described that melanoma cell lines express no or low surface, but significant intracellular levels of HLA-E inducible by IFN-γ, and some of them produced a soluble form of this molecule. In addition, according to these authors, a majority of tumour cells of primary melanoma, but a low proportion of metastatic melanomas (30–70% and 10–20%, respectively), expressed HLA-E [10].

We compared the distribution of HLA altered phenotypes established in the ESTDAB melanoma cell lines with HLA expression patterns previously found in melanoma primary lesions or other types of human tumour tissue by

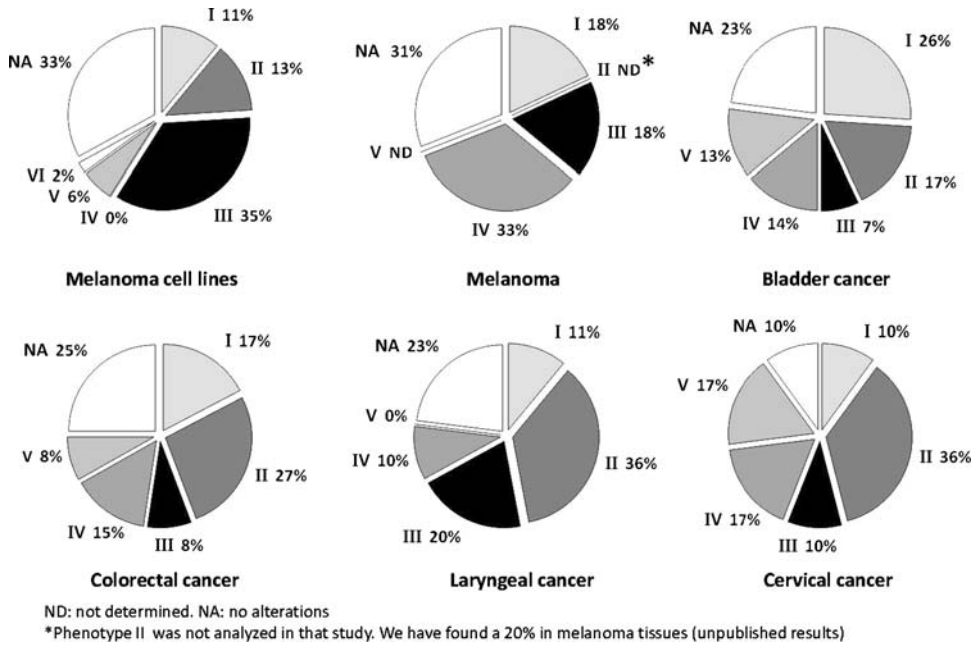

Fig. 1 Distribution of HLA class I phenotypes in 91 human melanoma cell lines (ESTDAB) and in different solid tumours. These tumours include melanoma [19], bladder cancer [30], colorectal carcinoma [29], laryngeal [28] and cervical cancer [22]. The following HLA class I expression phenotypes are compared: total loss or downregulation of HLA class I (Phenotype I); haplotype loss (Phenotype II); locus downregulation (Phenotype III); allelic loss (Phenotype IV), compound phenotype (Phenotype V); resistance to stimulation with IFN-gamma (Phenotype VI). Surface expression of HLA class I molecules in ESTDAB melanoma cell lines was determined by flow cytometry (mean fluorescence intensity, MFI) using anti-HLA class I specific antibodies. To analyse induction with IFN-gamma the melanoma cells were treated with 800 U/ml for 48 h

immunohistological studies (Fig. 1) [19, 22, 28–30]. For example, in cervical and laryngeal cancers the Phenotype I has similar incidence (about 10%) to the studied melanoma cell lines [22, 28]. In colorectal cancer, melanoma tumours and bladder cancer, it has higher occurrence, 17, 18 and 26%, respectively [19, 29, 30]. It is impossible to distinguish Phenotypes Ia and Ib in tumour tissues because only immunohistological analysis without molecular assays does not give information about the degree of HLA downregulation and whether the underlying alterations are structural or reversible in nature. Phenotype II was found in 13% of the studied melanoma cell, while in various types of solid tumour it has been reported with higher frequency ranging from 17 to 36% (Fig. 1). According to our results, Phenotype III (HLA locus downregulation) is the most frequent phenotype in the studied human melanoma cell lines (35%). In melanoma tissues, this phenotype was found in 18%, while that in laryngeal carcinoma was reported in 20% of the tumours, and in colorectal, bladder and cervical carcinomas only in 7–10% of the cases. Importantly, in melanoma cell lines the locus-specific downregulation affected only the B locus, while none of the cell lines had decreased expression of locus A. This suggests a possibility that locus B might be playing a preferential role in presentation of peptides derived from melanoma TAAs. In all melanoma cell lines analyses, the expression of locus B was recovered by IFN-γ, indicating that structural defects are not implicated. The frequency of Phenotype IV established in the studied melanoma cell lines cannot be compared with other studies because the expression of single specific HLA class I alleles was analysed in a limited number of melanoma cell lines due to the low number of HLA allele-specific antibodies available for flow cytometry. Phenotype V, a compound phenotype, found in 6% of the studied melanoma cell lines, was reported with higher incidence in bladder and cervical cancer, in 13 and 17% of cases, respectively, and without any occurrence in laryngeal carcinoma. Phenotype VI cannot be detected in solid tumours by immunohistological analysis. In our study it was found only in two cell lines indicating that alteration of IFN-gamma transduction signal pathway is not a frequent event in melanoma cell lines [48]. The percentage of melanoma cell lines without alterations in HLA molecules expression (33%) was similar to melanoma tissues and higher than in other types of tumour (Fig. 1). According to this figure, the overall percentage of HLA class I abnormalities among tumour with distinct histology is equally high, ranging from 67% depending on the type of tumour. However, the distribution of various HLA class I altered phenotypes and the underlying molecular mechanisms vary among the malignancies. These differences are likely to produce distinct routes of tumour immune escape and may influence the

prognosis and clinical course of various types of cancer. For instance, loss of HLA class I expression is often associated with poor prognosis in many types of cancer [2, 18, 35, 36, 38, 51, 56]. According to a recent study, a downregulation of HLA class I in rectal cancer is also associated with poor prognosis [53]. On the contrary, in colorectal cancer just a reduced HLA class I expression was reported to be associated with worse prognosis than a total class I loss or a high expression [57], likely due to the immune escape from both NK- and T-cell-mediated immune surveillance. The discrepancy between these reports might be explained by the fact that a large proportion of HLA negative colon tumours have microsatellite instability (MSI) which is associated with better prognosis. Therefore, characterizing the molecular mechanisms underlying HLA class I defects in tumour cells is important to predict the clinical course of cancer and to design the most advantageous treatment protocols.

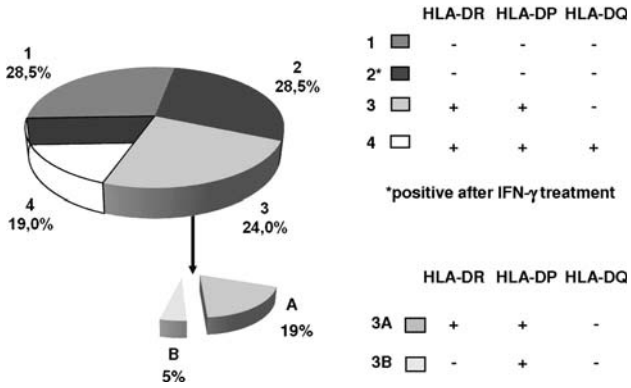

Fig. 2 Distribution of HLA class II phenotypes in 42 human melanoma cell lines (ESTDAB). Phenotypes 1–4 representing various patterns of HLA class II expression on the studied melanoma cell lines are presented. Surface expression of HLA class II molecules was determined by flow cytometry (mean fluorescence intensity, MFI) using a panel of HLA class II specific antibodies. To analyse induction with IFN-gamma the melanoma cells were treated with 800 U/ml for 48 h

HLA class II expression in ESTDAB melanoma cell lines

In the absence of inflammation, expression of MHC class II molecules is mainly restricted to cells of the hematopoietic system and thymus epithelium [45]. Nevertheless, a positive expression of HLA class II antigens have been detected in various types of solid tumour [1, 6]. Moreover, as tumour incidence is often accompanied by inflammatory events, where cytokines as IFN-γ are released, it has been reported that class II molecules might be induced in vivo in tumour cells [16, 24]. We analysed constitutive and IFN-γ-induced HLA class II expression in 42 human melanoma cell lines form the ESTDAB cell bank and based on our findings [49] were able to define four different HLA class II tumour phenotypes (Fig. 2): (a) Phenotype 1, negative HLA class II expression before and after treatment with IFN-γ, representing a 28.5% (12 cell lines); (b) Phenotype 2, absence of constitutive HLA class II expression, with some HLA class II isoforms induced by IFN-γ (12 cell lines, 28.5%); (c) Phenotype 3, positive constitutive expression of certain HLA class II isoforms, namely, 19% (8 cell lines) were positive for HLA-DR and -DP, and a 5% (2 cell lines) were positive only for HLA-DP; and (d) Phenotype 4, constitutive surface expression of all three class II isoforms (HLA-DR, -DP, and -DQ), representing 19% of the studied cells (8 cell lines) (Fig. 2) [49]. In all melanoma cells with positive constitutive expression of some HLA class II molecules (Phenotypes 3 and 4), this expression was enhanced by IFN-γ treatment. As far as we know, this is the first time that different phenotypes of MHC class II expression are defined in detail in human melanoma cell lines. In general, previous reports have showed HLA class II expression in melanoma without defining a specific locus. We found that 24% of the studied melanoma cell lines express constitutively HLA-DP or/and HLA-DR locus, and that HLA-DQ is present only when all other isoforms are expressed. The results show that in 71.5% of the cases (in 30 melanoma cell lines) there is a positive constitutive- or induced-surface expression of at least one HLA class II locus: 43% of constitutive expression and 28.5% IFN-γ-induced expression. Similar results on HLA class II constitutive expression were reported in a study with 85 melanoma cell lines from American Joint Committee of Cancer, where high levels of constitutive HLA class II expression was found in approximately a 40% of the studied cell lines [2]. However, in this study, neither differential locus expression nor IFN-γ-induced expression was analysed. In other types of malignancy, such as renal carcinoma and colorectal carcinoma, the HLA class II antigens are also frequently expressed de novo [9, 17, 34]. Moreover, in colon and renal cell carcinoma, a large number of HLA class II presented peptides, some containing ligands from several TAAs, have been detected [9, 26].

The high percentage of melanoma cells with positive constitutive or induced HLA class II expression suggests that these molecules might play an important role in melanoma. Several TAA-derived peptides that are presented by HLA class II molecules and recognized by CD4+ T lymphocytes have been identified in melanoma [21, 23, 31, 41, 47, 50]. In general, de novo expression of class II molecules loaded with new peptides should increase the immunogenicity of tumour cells. However, it has been also described that melanoma cells expressing HLA class II molecules induce the secretion of immunosuppressive cytokine IL-10 by T cells, promoting T cell-anergy [5]. Contradictory results have been reported on the association

between disease prognosis and HLA class II expression of tumour cells. Early publications on patterns of HLA class I and II expression in metastatic melanoma reported that high expression of HLA class II antigens might be associated with shorter patients' survival [55]. On the contrary, more recent analysis of HLA class II expression in melanoma, showed the significant association of class II expression with longer survival of patients [2]. These results are in agreement with the results obtained in large B-cell lymphoma and colorectal carcinoma [34, 46].

Correlation of HLA class I and II expression in ESTDAB melanoma cell lines

To get a total picture of the antigen-presenting ability of the studied melanoma cells, we analysed and summarized in Table 1 the association between HLA class II and class I expression on these cells. First, we classified the studied cell lines according to HLA class II phenotype, and then correlated these results with class I expression. As a result, we defined four groups of cell lines. In group "a", the melanoma cells are totally negative for HLA class II expression and negative or weakly positive for HLA class I expression. The groups "b" and "c" display melanoma cell lines with a variable degree of a positive expression of HLA class I and II. In group "d", all melanoma cell lines present high expression level of both HLA class I and class II antigens, and in this group does not include cell lines with negative HLA class I expression. The HLA class I and II expression increases from group "a" to group "d". Notably, always a positive constitutive expression of HLA class II expression coincides with some level of HLA class I expression in melanoma cell lines. A positive correlation between HLA class I and class II expression has been also reported by other groups [2]. Together with our data, this suggests the presence of a common regulatory mechanism of class I and II expression in melanoma cells [54]. Class II positive melanoma cells present a more immunogenic phenotype with a possibility to load TAA-derived peptides via MHC class I and II molecules, that can be recognized by both CD8+ and CD4+ T lymphocytes, respectively. On the other hand, melanoma cell lines with absence of class II expression have negative or weakly positive HLA class I phenotype with low antigen presentation capacity (Table 1). In cutaneous melanoma, tumour cells with such phenotype were reported to be associated with poor prognosis [2, 18, 56].

The analysis of the HLA expression patterns (Table 1) and the underlying molecular mechanisms of HLA alterations in the studied melanoma cell line collection may help to select particular cell lines for further detailed study. For example, in the group "a" there are two cell lines, ESTDAB-038 and ESTDAB-109, which do not present neither constitutive nor IFN-γ-induced surface expression of HLA class I and class II molecules. Mutations in beta-2 microglobulin gene are responsible for a total loss of HLA class I expression in these cell lines [4]. These two melanoma cell lines cannot present antigens and are totally invisible to T-lymphocytes. Other two cell lines from the same group "a", ESTDAB-004 y ESTDAB-159, have low level of HLA class I expression, which is not inducible with IFN-γ due to defective STAT-1 mediated signal transduction [48]. This group also includes three cell lines, ESTDAB-127, ESTDAB-094, ESTDAB-102, with total HLA class I downregulation (Phenotype Ib) associated with low expression of the components of the antigen processing machinery, TAP-2 y LMP-7 [37]. In the future, other melanoma cell lines may be selected to study the molecular mechanisms implicated in HLA class I or II expression.

Concluding remarks

The European Searchable Tumour Line Database (ESTDAB) Cell Bank is a collection of immunologically characterized human melanoma cell lines. Here, we reviewed the HLA class I and II phenotypes found in the ESTDAB melanoma cell lines, classifying them into different groups, and compared these data with those described in other types of malignancy. The analysis of the HLA expression in the ESTDAB melanoma cell lines gives to the scientific community an opportunity to use in their research projects melanoma cell lines with well characterized HLA class I and II phenotypes. Identification and characterization of cancer-associated genes, proteins and biomarkers that are associated with malignant transformation may help to understand the mechanism of cancer pathogenesis and progression, and to predict and explain the responsiveness to treatment. HLA class I and II molecules might be considered as a cancer biomarker since its altered expression has been found in many types of cancer and in many cases it is associated with metastatic progression and poor prognosis. Numerous HLA binding peptides derived from tumour-specific antigens have now been identified, and many have been used in clinical trials using peptide-based vaccines. In addition, more traditional cancer therapy treatment, such as chemotherapy, also promotes TAA-peptide specific anti-tumour reactivity by killing immunogenic tumour cells and subsequent release of tumour associated peptides and proteins. In this case, the lack of functional HLA antigens would limit the positive outcome of such immunostimulation. Moreover, recent findings indicate that HLA class I molecules may have direct implications in other biological process, such as resistance to apoptosis [52, unpublished observations]. Therefore, analysis of the tumour expression of HLA class I and II antigens might help to choose an appropriate

Table 1 HLA class I and II surface expression in melanoma cell lines from ESTDAB

Cell lines	HLA CLASS II						HLA class I	Phenotype
	Basal			IFN-γ			Basal	
	DR	DP	DQ	DR	DP	DQ	ABC	
Group a (28.5%)								
ESTDAB-038	–	–	–	–	–	–	–	Phenotype Ia[b]
ESTDAB-109	–	–	–	–	–	–	–	Phenotype Ia[b]
ESTDAB-127	–	–	–	–	–	–	+	Phenotype Ib
ESTDAB-094	–	–	–	–	–	–	+	Phenotype Ib
ESTDAB-102	–	–	–	–	–	–	+	Phenotype Ib
ESTDAB-081	–	–	–	–	–	–	±	Phenotype V
ESTDAB-195	–	–	–	–	–	–	+	Phenotype V
ESTDAB-004	–	–	–	–	–	–	+	Phenotype VI[b]
ESTDAB-159	–	–	–	–	–	–	+	Phenotype VI[b]
ESTDAB-048	–	–	–	–	–	–	++	Phenotype III[a]
ESTDAB-179	–	–	–	–	–	–	++	Phenotype II
ESTDAB-049	–	–	–	–	–	–	+	Phenotype III[a]
Group b (28.5%)								
ESTDAB-140	–	–	–	+	–	–	+	Phenotype Ib
ESTDAB-073	–	–	–	+	–	–	+	Phenotype Ib
ESTDAB-196	–	–	–	+	+	–	+	Phenotype Ib
ESTDAB-020	–	–	–	+	+	–	+	Phenotype Ib
ESTDAB-200	–	–	–	+	+	–	++	Phenotype III[a]
ESTDAB-199	–	–	–	+	+	–	++	Phenotype III[a]
ESTDAB-084	–	–	–	+	+	–	+++	Phenotype III[a]
ESTDAB-110	–	–	–	+	+	–	++	No alterations detected
ESTDAB-112	–	–	–	+	+	–	++	No alterations detected
ESTDAB-146	–	–	–	+	+	–	+++	No alterations detected
ESTDAB-069	–	–	–	+	+	–		
ESTDAB-162	–	–	–	+	+	+	+	Phenotype V
Group c (24%)								
ESTDAB-070	–	+	–	+	++	–	++	Phenotype II
ESTDAB-133	–	+	–	+	++	–	++	Phenotype II
ESTDAB-071	+	+	–	++	++	–	++	Phenotype II
ESTDAB-041	+	+	–	++	++	–	++	Phenotype II
ESTDAB-058	+	+	–	++	++	–	++	Phenotype II
ESTDAB-206	+	+	–	++	++	–	++	Phenotype III[a]
ESTDAB-074	+	+	–	++	++	–	++	Phenotype III[a]
ESTDAB-184	+	+	–	++	++	–	+++	No alterations detected
ESTDAB-153	+	+	–	++	++	–	+++	No alterations detected
ESTDAB-129	+	+	–	++	++	++	++	Phenotype II
Group d (19%)								
ESTDAB-137	+	+	+	+	++	++	+	Phenotype V
ESTDAB-167	+	+	+	++	++	++	+++	Phenotype II
ESTDAB-130	+	+	+	++	++	++	+++	Phenotype II
ESTDAB-108	+	+	+	++	++	++	+++	Phenotype III[a]
ESTDAB-152	+	+	+	++	++	++	+++	Phenotype III[a]
ESTDAB-183	+	+	+	++	++	++	++	Phenotype III[a]
ESTDAB-016	+	+	+	++	++	++	+++	No alterations detected
ESTDAB-136	+	+	+	++	++	++	+++	No alterations detected

[a] HLA-B downregulation

[b] No response to IFN-γ treatment

treatment protocol and monitor clinical response to cancer therapy.

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