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Biomarkers in chronic graft-versus-host disease - quo vadis?

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Abstract

Biomarkers are increasingly used for diagnosis and treatment of transplant related complications including the first biomarker-driven interventional trials of acute graft-versus-host disease (GvHD). In contrast, the development of biomarkers of chronic GvHD (cGvHD) has lagged due to a broader variety of manifestations, overlap with acute GvHD, a greater variability in time to onset and maximum severity, and lack of sufficient patient numbers within prospective trials. An international workshop organized by a North-American and European consortium was held in

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Marseille in March 2017 with the goal of identifying strategies for future biomarker development to guide cGvHD therapy. As a result of this meeting, two areas were prioritized: the development of prognostic biomarkers predicting the subsequent onset of moderate/severe cGvHD, and in parallel, the development of qualified clinical grade assays for biomarker quantification. The most promising prognostic serum biomarkera are CXCL 9, ST2, matrix metalloproteinase-3, osteopontin, CXCL 10, CXCL 11 and CD163. Urine-proteomics and cellular subsets ($CD4^+$ T cell subsets, $co21^{low}$ B cells) represent additional potential prognostic biomarkers of cGvHD. A joint effort is required to verify the results of numerous exploratory trials before any of the potential candidates is ready for validation and subsequent clinical application.

Keywords

allogeneic hematopoietic stem cell transplantation; biomarker; chronic GVHD

Introduction

Biomarkers are increasingly considered in the treatment of malignant diseases and are currently being validated with regards to outcome prediction in patients with acute graft-versus-host disease (aGvHD).[1, 2] The first trials have been launched where biomarker assessment determines aGvHD immunosuppressive interventions through the Blood and Marrow Transplant Clinical Trials Network (BMTCTN protocol 1501).[2] Despite the increasing importance of chronic GvHD (cGvHD)[3], the identification of biomarkers in cGvHD has lagged due to several factors. These factors include a) a broad variety of manifestations which drive prognosis and reflect a potentially heterogeneous pathophysiology including overlap with aGvHD, b) a much longer time frame trajectory, and c) a lack of sufficient patient numbers within multicenter trials to adjust for the heterogeneity of patients with cGvHD. The NIH consensus on cGvHD in 2005 provided the basis for dragnustic criteria and biomarker development.[4, 5] These consensus recommendations were updated in 2014[6, 7] defining the steps for exploration (identification of potential biomarker candidates) and verification of potential biomarkers (replication in independent cohorts including test practicability and stability) prior to qualification for clinical application. Currently, the development of biomarkers has not passed the verification phase mainly due to three reasons. First, the evaluated biomarkers differed in trials due to technical reasons and selection of different probes and time points (before or after start of immunosuppression) making it currently impossible to select an optimal biomarker panel to be validated. Second, cGvHD biomarker studies try to predict development of any cGvHD without recognizing subgroups that may have different pathogenesis. Third, heterogeneity in the laboratory assays not approved for clinical use could be causing significant variation of results in verification trials. For example, CXCL 9 has been identified within several cohorts[8, 9] as the most sensitive marker while within other cohorts, CXCL 10 performed better compared to CXCL 9.[10, 11] An additional issue relates to the need for prospective documentation of cGvHD at the time of clinical assessment within verification trials. This is important, since any retrospective documentation bears the risk of insufficient clinical details or possible retrospective bias in severity interpretation with the knowledge of prolonged follow up after the sample was obtained. In summary, currently some serum/plasma

biomarkers[9, 10] and to a lesser extent cellular subpopulations[12] represent the most promising markers. Urine proteomics may be a potential option[13] while gene expression assays require further exploration. Besides HLA-typing, none of the genetic markers reached an association level sufficient to be useful as a biomarker of cGvHD in a clinical setting.[14]

To improve the current situation an international workshop on the development of biomarkers was organized by a North-American and European consortium which was held in Marseille on March 24th and 25th 2017. The workshop included a critical review of current evidence and sought to develop strategies for future joint efforts towards qualified biomarker development for guidance of cGvHD therapy. The workshop summary is presented here.

Consideration of clinical heterogeneity

Development of biomarkers in cGvHD may require some specific consideration with regard to the sensitivity and specificity in subgroups with different clinical characteristics. Any biomarker should be carefully evaluated during the verification phase with regard to cofactors that may affect biomarker levels. These factors for instance can include donor source, intensity of the preparative regimen or the use of total body irradiation.[6, 10] The inclusion of control patients after autologous peripheral stem cell transplantation may help to adjust for non-cGvHD confounding factors. Moreover, detection and impact of specific organ patterns of cGvHD should be evaluated. An additional issue is understanding the contribution of concurrent acute GvHD (called overlap-subtype of cGvHD) which may result in misattribution of late acute GvHD biomarkers to cGvHD. Additional crucial issues are the impact of immunosuppression and concurrent infections on biomarker levels, as for instance steroids suppress sBAFF[15] while viral infections with herpes class viruses like CMV may induce CXCL 10 expression.[16]

Development of a clinical grade assay and pre-requisites of validation trials

An additional general issue is that none of the currently applied assays is approved for clinical use so qualification of a biomarker requires in parallel a verified diagnostic tool as outlined in the 2014 NIH consensus on cGvHD[6]. As a result, the impact of numerous covariates summarized in[6] and confirmed in[10] require standardized documentation within future biomarkers-trials including time after transplantation, stem cell source, immune reconstitution, prior acute GvHD, intensity of immunosuppression at time of assessment and before assessment, and the presence of infections. Sampling of biomarker probes requires application of standard operating procedures (SOPs) focused on the collection, transportation, and processing of samples. Biomarker terminology also needs to be standardized. To harmonize with concepts used by the US Food and Drug Administration (FDA)[17] the following terminology was proposed in the context of cGvHD: *prognostic* biomarkers aim to provide information about the risk for subsequent cGvHD while *predictive* biomarkers are applied to predict the course of cGvHD at diagnosis or at later timepoints. *Diagnostic* biomarkers are used to confirm the diagnosis of cGvHD.[6]

With regard to qualification of biomarker for clinical application two components are to be considered: 1) assay qualification (which may be performed on a retrospective cohort) and

2) clinical qualification in prospective cohorts and assessment in utility trials. As already mentioned standardized assays with standardized SOPs must be verified as well to allow for reproducibility in the qualification studies. To address clinical qualification, the workshop participants agreed on the need for a prospective multicenter cohort including prospective documentation of clinical data (outlined in detail in[6]). as well as standardized sampling in newly diagnosed, untreated patients. Potential markers to be evaluated are outlined below.

Identification of prognostic biomarkers

Prognostic biomarkers predict the future development of cGvHD. The group felt that it is most important to prognose the development of moderate-severe forms of cGvHD to target for prevention, since mild cGvHD does not cause morbidity and mortality and is associated with superior overall survival due to a graft-versus-tumor effect and prevention of mild cGvHD could paradoxically worsen outcomes.[18, 19] Therefore, any prognostic biomarker should have a high negative predictive value to avoid inappropriate prolonged immunosuppression, while impaired positive predictive value is less problematic because at worst a patient would start treatment of cGvHD once symptoms appear which is currently standard management. Potential serum candidates are CXCL 9[20], ST2, matrix metalloproteinase-3, osteopontin[9], CXCL 10, CXCL 11,[20, 21] and CD163 (plasma).[22] Urine proteomics may be an additional prognostic approach since it showed a sensitivity and specificity of 84% and 78% respectively within 2 separate European cohorts but failed to correlate with current cGvHD in an US cohort (Lee, S.J. verbal communication). With regard to cellular markers the expansion of naYve $CD4^+$ T cells as well as $CD21^{low}$ B cells may serve as prognostic biomarkers of subsequent cGvHD taking into account that $CD21^{low}$ B cells consist of at least 3 different subpopulations and it is currently unclear which of these is most relevant[12, 21, 23] Additional cellular subsets including regulatory T cells, regulatory NK cells, and NKT cells are currently being explored but require further evaluation.[24, 25], With regard to genetic biomarkers, a number of candidates of prognostic polymorphisms were identified in small and medium sized cohorts but failed to be consistently replicable in larger cohorts underlining the crucial role of sufficiently powered replications sets including different donor types and graft sources.[14] A summary of trials evaluating prognostic, diagnostic and predictive markers is shown in table 1.

Identification of diagnostic biomarkers

While cGvHD is usually easily diagnosed based on clinical and histological criteria[7, 26, 27] certain clinical conditions may benefit from biomarker measurements. For example, a considerable portion of patients may be thought to have cGvHD but do not show diagnostic signs 7 and require histopathological confirmation which may be invasive (liver, lung)[26, 28], or difficult to obtain and interpret (eye) or be time consuming.[29] In addition, some pediatric evaluations are particularly challenged when testing cannot be performed (i.e., pulmonary functions testing and Schirmer's test) and histopathology requires general anesthesia. Therefore, an easy to assess biomarker would be a significant advantage in clinical care by speeding up the diagnostic evaluation, providing additional certainty of the diagnosis and may furthermore serve a quality control purpose for inclusion in clinical trials. Last but not least, organ specific biomarkers may help to differentiate active organ involvement caused by GvHD from other organ impairments caused by comorbidities like preexisting chronic

obstructive pulmonary disease. A number of serum/plasma candidate proteins have been explored as diagnostic biomarkers of cGVHD. CXCL 9, CXCL 10, and sBAFF have been most frequently associated with the onset of cGvHO,[8–10, 30, 31] with anti-LG3, ST2, matrix metalloproteinase 3, and osteopontin being additional candidates.[9], 10 With regard to cellular biomarkers a high proportion of $co19^{+}co21^{low}$ B cells and $CD4^{+}CD45RA^{+}CD31^{+}$ T cells have been associated with diagnosis of cGvHD[12, 32] while within a different cohort the lack of $CXCR3^{+}$ (ligand for CXCL 9 &10) $CD56^{bright}$ NK cells correlates with diagnosis of cGvHD with $CXCR3^{+}CO4^{+}$ T cells being an additional cellular marker of interest[10] while other cellular subsets like regulatory T cells and their ratio to effector T cells require further evaluation.[24] With regard to organ specific biomarkers very high sBAFF levels and expansion of $co21^{low}$ B cells have been associated with lung manifestations.[32] In acute GvHD, elafin is a specific marker for skin involvement[33, 34] and Reg3alpha indicates gastrointestinal manifestations and could indicate overlap cGvHD.[35] Currently, organ specific serum marker for oral or ocular GvHD are lacking but saliva[36] or tear proteomics[37] have been explored.

Identification of predictive biomarkers

Limited data are restricted to exploratory studies showing that normalization of sBAFF and regeneration of naïve B cells after exposure to rituximab are associated with response to B cell depletion[38, 39] and ECP.[40] Moreover, persisting CXCL 10, CXCL9 and ST2 has been associated with active cGvHD although additional studies are needed.[30] An additional aspect of predictive biomarkers is the differentiation of non-reversible inactive lesions of cGvHD from active disease which may be relevant during the course of disease.

Additional biomarkers have been evaluated in different indications recently summarized in[6] and further insight in the pathophysiology of cGvHD was recently summarized in[24].

Future collaboration

The first level of qualification requires development and application of qualified assays to guarantee that measurement of biomarkers is consistent between laboratories. In parallel, the development of certified assays in cooperation with industrial partners for clinical application is of crucial relevance, since none of the currently applied methods has been certified for clinical application which is the pre-requisite for qualification within clinical trials. Since it is unlikely, that a single biomarker will be sufficient to cover all aspects of cGvHD, it will be important to define a panel of biomarkers before developing clinical grade assays as multiplex ELISA’s are technical challenging and the development of assays using direct detection of proteins or protein fragments require a pre-defined panel to be cost effective. While assay qualification may be based on already existing samples clinical qualification requires a prospective sampling.

First steps will be the implementation of prospective biomarker trials within the US cGvHD-consortium, the Canadian pediatric ABLE-consortium and the German-Austrian-Swiss GvHD consortium including standardized sample preparation, and storage, exchange of reference samples and protocols between the centers to test comparability of results followed by a prospective cohort study, with internal standards and multiple measures conducted in all

participating laboratories. The verification and validation of candidate biomarkers in pediatric populations is highly relevant since this is a notoriously underrepresented population within clinical trials and adult data may not be extrapolated to the pediatric population. In summary a joint effort is required to verify the results of numerous exploratory trials before any of the potential candidates is ready for validation and subsequent clinical application.

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Table 1

Published evidence for biomarker candidates In cGvHD (only trials including > 70 patients are listed)

Number of patients	Marker				Ref
	Type	Source[1]	Use	Name	
Major Pediatric Studies					
81	Pl	R	D	IL-2Rα, sBAFF, sCD13, anti-dsDNA	41,42
	C	R	D	CpG responsive B cells, IFNγ^+ and IL-2^+ T cells	43, 44
	Pl, C	R	Pre, RT	sBAFF, CoG resoonsive B cells	43
Major Adult studies					
349	Pl, C	R	D	sBAFF, CXCL 10, CXCL9, CXCL 11, slL2Ralpha, sCD13, ICAM1, anti-LG3, endothelin-1, CXCR3^+ NK_{reg} cells	10
107	C	Do	Prog2	$CD56^{bright}$NKreg cells, IFNγ^+ $CD4^+$ T cells	25
320	Pl	R	D	sBAFF. CXCL 10, CXCL9, slL2Rα, sCD13, sST2	8
391	Pl	R	Prog1	CXCL9. sST2. Metalloprotease 3, Osteoponin	9
70	C	R	D	$CD21^{low}$ B cells- and lack of memory $CD27^+$ B cells, $CD4^+CD45R^+$:$CD4^+CD45RA^+CD31^+$ T cells ratio, $CD56^+$:$CD3^-CD56^{high}$ NK ratio, BAFF/$CD19^+$:$CD19^+CD21^{low}$B cells	45
163	C, Pl	R	Prog1	$CD19^+CD21^{low}$ B Cells, $CD4^+CD45RA^+CD31^+$ T Cells, $CD56^{bright}$ NK cells	12
115	Pl	R	D	CXCL 10, CXCL 11, sBAFF	31
107	C	R	Prog1	Central memory:effector memory T cell ratio, regulatory T cells/conventional T cell ratio	46
104	Pl, C	R	D	sBAFF and B cells	15
489	u	R	Prog3	cGvHD_MS14 classifier includes 14 proteins	13
167	Pl	R	Prog1	CD163	22
92	R	R	D	IRS2, PLEKHF1 and IL1R2 plus CMV status and conditioning regimen intensity	47
3918	R	R	Prog2	Polymorphisms of CTLA4, HPSE, and IL1R1 were identified in the first cohort but could not be validated	14

D = diagnostic, Prog1 = Prognostic measured at day 80–100, Prog2= Prognostic measured in donor product at time of transplant, Prog3 measured up to 55 days before diagnosis; R=response to therapy, Pre= Predictive biomarker; Pl = plasma, C = cells, R = RNA, U = urine

[1]Source of the biomarker RT= recipient after BMT, Do= donor cell product at time of BMT