efforts applying massively parallel sequencing methods had focused on examining so-called index patients to investigate the landscape of molecular mutations in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). These studies led to the discovery of key mutations in genes such as *IDH1, DNMT3A, BCOR, RAD21*, and unraveled the involvement of the dysregulated splicing machinery in various types of hematological malignancies *(SF3B1, SRSF1, U2AF1, ZRSR2)*. Consequently, molecular biomarkers will soon no longer be sequenced individually. Instead, panels of markers will be assessed in a massively parallel way with high sensitivity and multiplexing many patients per analysis. A great challenge will be the implementation of such a novel methodology into existing laboratory workflows. Here, we present potential applications of this assay and in particular will focus on the utility of amplicon deep-sequencing in characterizing myeloid and lymphoid neoplasms where the number of molecular markers applied for disease classification, patient stratification into differing risk groups, and individualized monitoring of minimal residual disease is constantly increasing. We will discuss many facets of this assay that need to be taken into account, e.g. the preparation of sequencing libraries with molecular barcodes, specific experimental design options when considering sequencing coverage to calculate diagnostic sensitivity, or the use of suitable software and data processing solutions to obtain accurate results. Taken together, amplicon deep-sequencing has already demonstrated a promising technical performance that warrants the further development towards a routine application of this next-generation sequencing technology in diagnostic laboratories so that an impact on clinical practice can be achieved. Besides being able to address the ever growing demands for high-throughput clinical research topics, NGS will be particularly useful to provide molecular information for many disease areas in the cost-effective manner and fast turn-around time that individualized treatment regimens will be requiring.

Abstracts oral presentations O.1 - O.8

O.1 ABVD (8 cycles) vs. BEACOPP (4 escalated cycles => 4 baseline) in stage III - IV low risk Hodgkin Lymphoma (IPS 0-2): final results of LYSA H34 trial

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Escalated BEACOPP achieved superior time to treatment to failure over ABVD in patients with disseminated Hodgkin lymphoma. However, later clinical trials have failed to confirm Overall Survival (OS) superiority over ABVD.

Methods

We compared ABVD (8 cycles) vs. BEACOPP (escalated 4 cycles => baseline 4) in patients with International prognostic score (IPS) ranging 0-2. Primary endpoint was Event Free Survival. Patients with IPS >2 were included in the EORTC Intergroup 20012 study.

Results

One hundred fifty patients were randomized (ABVD 80, BEACOPP 70): median age 28 y, males 50%. IPS was 0-1 for 64%. CR was 85% for ABVD and 90% for BEACOPP. Progression or relapses were more frequent in ABVD (17 vs 5 patients). With a median follow-up of 5.5 years, 7 patients died : 6 in ABVD and 1 in BEACOPP (HL 3 & 0, 2nd cancer 2 & 1, accident 1&0). EFS at 5 yrs was estimated at 62 % vs. 77 %, respectively (HR = 0.6, p=0.07). Progression Free Survival at 5 yrs was 75 % vs. 93 % (HR = 0.3, p=0.007). Overall suvival at 5 yrs was 92 vs. 99 % (HR = 0.18, p=0.06).

Conclusion

EFS and OS were not different between treatment arms. However, more progressions/relapses were observed with ABVD. As in high risk group, additional considerations as late morbidity due to salvage treatment may help decisions making toward ABVD or BEACOPP for low risk patients.

O.2 Establishment of a murine graft-versus-myeloma model using allogeneic stem cell transplantation

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Multiple myeloma (MM) is a malignant plasma cell disorder with poor long-term survival and high recurrence rates. Despite evidence of graft-versus-myeloma (GvM) effects, the use of allogeneic stem cell transplantation (allo-SCT) has remained controversial in MM. In the current study, we investigated the anti-myeloma effects of allo-SCT from B10.D2 mice into MHC-matched myeloma-bearing Balb/cJ mice (previously injected with the MOPC315.BM myeloma cell line), based on a chronic graft-versus-host disease (GvHD) murine model.

Balb/cJ mice were injected intravenously with luciferase-transfected MOPC315.BM cells, and received 30 days later an allogeneic (B10.D2 donor) or autologous (Balb/cJ donor) transplantation by intravenous administration of bone marrow cells and splenocytes. We observed a graft-versus-myeloma effect in 17 out of 18 allogeneic transplanted mice, as luciferase signal completely disappeared after transplantation, whereas 13 of the 13 autologous transplanted mice showed myeloma evolution. Lower serum paraprotein levels and myeloma infiltration in bone marrow and spleen in the allogeneic setting confirmed the observed GvM effect, while allogeneic mice also displayed chronic GvHD symptoms. Moreover, prior sensitization of B10.D2 donor mice with myeloma cells resulted in exacerbated GvHD symptoms in recipient mice, suggesting a cross-reactivity of responses directed against antigens present on myeloma cells and allo-antigens. *In vivo* and *in vitro* data suggest possible involvement of effector memory CD8 T cells in the GvM effect, reactive against both myeloma and normal Balb/cJ cells. Finally, the role of CD8 T cells was confirmed when CD8 T-cell depletion of the graft resulted in reduced GvM effects. In the CD8 T cell-depleted group, 4 out of 6 mice (66.7 %) showed strong bioluminescence signal and myeloma symptoms after transplantation, whereas in the standard allogeneic transplantation group, only one mouse out of 18 developed bioluminescence signal after transplantation (5.6 %).

We successfully established an immunocompetent murine graft-versus-myeloma model, involving effector memory CD8 T cells which display both anti-myeloma activity and alloreactivity. This model could be a basis for further studies assessing ways of dissociating GvM from GvHD.

O.3 Comparison of 2 nonmyeloablative regimens for allogeneic HCT: a phase II randomized study from the HCT committee of the BHS

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