with antibodies targeting CD33, CD123 und CSF1R achieved efficient *in vitro* activation, cytotoxicity and cytokine production against tested AML cell lines and primary AML blasts, while antigen-negative ALL cell line NALM-6 was not killed. Remarkably, effector functions of anti-P329G CAR T cells were similar to classical CAR T cells particularly when targeting CD33. Anti-P329G CAR T cells activated by recombinant protein showed no cytokine production in the presence of an antibody which was not recognizing the respective antigen. However, by switching the antibody after 24 h of stimulation towards the tumor antigen-targeting antibody, CAR T cell activation could be again achieved by the recombinant protein. Depleting the tumor antigen-targeting antibody after 24 h of stimulation decreased the cytokine production after 48 h and 72 h despite ongoing presence of the recombinant protein. This confirms the modularity and reversibility of this adaptor CAR T cell platform. CAR T cells combined with a CD33-targeting antibody showed efficient tumor clearance of THP-1-bearing immunodeficient mice.

Conclusions Taken together, anti-P329G CAR T cells combined with Fc-silenced tumor antigen-targeting IgG1 antibodies carrying the clinically validated PGLALA-mutations in the Fc part achieved profound effector functions against various human AML cell lines and primary AML blasts. The modular platform has the potential to overcome certain limitations of CAR T cell therapy in AML.

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P12.03 HETEROTYPIC CD8 T CELL CLUSTERS ISOLATED FROM CLINICAL SAMPLES ARE DISTINCT AND ENRICHED FOR ANTITUMOR ACTIVITY

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Background An increasing body of evidence suggests that in addition to the type, density, and state of immune cells in the tumor microenvironment (TME), also their proximity to cancer cells influences immunotherapy outcome. For example, favorable responses to immune checkpoint inhibitors in melanoma are associated with higher densities of $CD8^+$ tumor-infiltrating lymphocytes (TIL) within 20 µm distance of melanoma cells. This notion is in line with the understanding that upon specific antigen recognition, cytotoxic T cells physically engage with their target cells through their TCRs followed by immunological synapse formation. Indeed, structural and functional avidity of cytotoxic $CD8^+$ T cells correlates strongly with their activity against cancer cells. Together, these observations point to the importance of direct interactions between cytotoxic T cells and tumor cells in the TME. This led us to investigate whether tumor-specific $CD8^+$ T cells can be isolated from clinical cancer specimens as heterotypic clusters.

Materials and Methods We employed a tumor cell-T cell co-culture *in vitro* model, patient samples and *ex vivo* assays. To evaluate functional interactions between human T cells and tumor cells, we made use of a system we engineered previously, comprising melanoma cells expressing both HLA-A*02:01 and the MART-1 tumor antigen. They were challenged with CD8+ T cells from PBMCs that were retrovirally transduced with a MART-1-specific TCR. To asses these interactions in patient material, upon surgical removal tissue was cut into small fragments, digested and analyzed by (image-based) flow cytometry. Interacting (cluster) and not-interacting (singlets) T cells were isolated and expanded *in vitro*. To characterize tumor cell:T cell interactions single cell TCR and RNA sequencing is used, as well as *ex vivo* co-cultures with autologous tumor cells.

Results We found that in defined co-cultures, tumor antigen-recognizing T cells were commonly enriched over non-specific T cells in heterotypic clusters with tumor cells, prompting us to investigate whether such specific clusters could be isolated also from cancer specimens. We observed that from 10/10 human melanoma metastases, we were able to isolate heterotypic clusters, comprising $CD8^+$ T cells interacting with one or more tumor cells and/or antigen-presenting cells (APCs), which was validated by imaging flow cytometry. Upon expansion, $CD8^+$ T cells from tumor cell clusters and APC clusters exerted on average 7.6-fold increased melanoma-killing activity over T cell singlets, which was associated with enhanced cytokine production. $CD8^+$ T cells from clusters were enriched for tumor-reactive and exhausted gene signatures. Integration with T cell receptor (TCR)-sequencing showed increased clonality of clustered T cells, indicative of expansion upon antigen recognition.

Conclusions Together, these results demonstrate that tumor-reactive CD8$^+$ T cells are enriched in functional clusters with tumor cells and/or APCs, and that they can be isolated and expanded from clinical samples. Being often excluded in cell sorting procedures, these distinct heterotypic CD8$^+$ T cell clusters serve as a valuable source amenable to deciphering functional tumor-immune cell interactions, while they may also be therapeutically explored.

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P12.04 ADHERENCE TO CLINICAL GUIDELINES FOR MANAGING FEBRILE NEUTROPENIA INPATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION: ARE WEINCLINED TO FOLLOW THE RECOMMENDATIONS OF IDSA AND NCCN IN LMIC?

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Background Severe infections and febrile neutropenia FN due to the administration of conditioningregimens are common among patients undergoing Bone marrow transplantation orHematopoietic stem cell transplantation HSCT. Compliance with the standard antibioticsguidelines like IDSA and NCCN in these patients is crucial to minimise the risk of prolonged-hospital stay, antibiotic resistance, increased pharmacoeconomic burden, morbidity andmortality. This study aimed to evaluate compliance with the FN clinical guidelines amongpatients undergoing HSCT.

Materials and Methods This is an ongoing prospective observational study is single-centre and was conducted at awell-known institute specialized for treating blood disorders and bone marrow transplantationfor 3 months. The recruited participants for inclusion criteria were those who at least had asingle episode of FN after post-HSCT. Antibiotics clinical guidelines compliance provided bythe Infectious Diseases Society of America (IDSA) and National Comprehensive CancerNetwork (NCCN) for FN treatment were assessed while reviewing patients' medical profiles.

Results The mean age of the patients was 15.09 years± 6.57, Till now in total 11 patients with 23episodes of FN were assessed. FN therapy with compliance-based guidelines (IDSA, NCCN)was examined in terms of selection, initial regimens and timing of antibiotics. Unfortunately,63% of recruited

Abstract P12.04 Table 1

Variables	(Mean ±SD), N (%)
Age	15.09 years± 6.57
Gender:	
Male	8
Female	3
Diseases leading to HSCT	
Beta Thalassemia Major	6 (54%)
Aplastic Anemia	1 (9%)
Relapse Hodgkin lymphoma	1 (9%)
Fanconis Anemia	1 (9%)
acute myelomonocytic leukemia	1 (9%)
MDS(RCC) Myelodysplastic Syndromes with Ringed Sideroblasts and Multilineage Dysplasia.	1 (9%)

Abstract P12.04 Table 2

HSCT Type	
Allogeneic	10 (90%)
Autologous	1 (10%)
Conditioning regimen	
Thiotepa,Cyclophosphamide,Busulphan,Antithymoglobin ,Fludarabine	1
Fludarabine,Antithymoglobin,Busulphan,Cyclophosphamide	7
BEAM	1
Busulfan+Cyclophosphamide	2

Abstract P12.04 Table 3

Document of infection	Frequency of patients
Microbiological document	
Blood culture	11
Urine Culture	11
Sputum Culture	NA
Hickman line culture	11
Clinical Signs	
Mucositis	4
Diarrhea	3
Catheter-related	1
Rediological evidence	
Chest X-ray	2
CT Scan	2