review and created a survey centered around the genetic testing practices and FISH report interpretation among MM clinicians. **Results:** Among 102 respondents, representing 14 countries, most clinicians (74%) utilized in-house FISH laboratories. Nearly all (90%) respondents desired FISH at diagnosis, 72% during disease progression, and 40% for treatment/response assessment. The most-requested probes included TP53 (99%), t(4;14) (92%), 1q gain/amplification (91%), t(14;16) (90%), t(11;14) (85%), t(14;20) (76%), 1p deletion (67%), while FISH for ploidy status, deletion 13q/-13, t(6;14), MYC rearrangement, and other rare IG rearrangements were ranked lower in importance (10-50%). Approximately 65% of respondents were satisfied with the clarity and interpretation of FISH reports. However, when challenged to interpret a particularly difficult FISH report, 20% responders interpreted results correctly while the majority were either unsure or misinterpreted the report. **Conclusions:** Our study highlights the need for significant improvements in MM FISH report clarity by the clinical laboratory directors to benefit both the clinician and patient. We propose solutions to improve standardization and recommend best MM FISH reporting practices that we aim will be adopted by clinical laboratory directors.

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Immunomodulatory Effects of Daratumumab on the Bone Marrow Microenvironment in Multiple Myeloma: Insights from Single-Cell Analyses

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Introduction: Daratumumab (Dara), a monoclonal antibody targeting CD38, is approved for treating multiple myeloma (MM). Its impact on the cellular immune compartment and the relation to response/resistance are not fully understood. Understanding Dara's effects on the immune landscape and its therapeutic mechanisms is crucial for identifying synergistic immunotherapeutic approaches and informing therapy sequencing for long-term efficacy. **Methods:** Mass cytometry (CyTOF) and single-cell RNA sequencing (scRNA-seq) were used to comprehensively characterize the immune microenvironment in MM patients undergoing Dara therapy, either as monotherapy or in combination regimens. CD138-negative fractions of bone marrow mononuclear cells (BMMCs) collected from MM patients at pre-treatment (pre-Dara, n=37), on-treatment (on-Dara; in remission, n=10), and post-treatment (post-Dara; relapsed, n=32) timepoints were analyzed to focus on the non-malignant immune compartment. **Results:** CyTOF and scRNA-seq analyses revealed a consistent decrease in the frequency of CD4+ T cells, B cells, plasma cells, and NK cells by up to 50%, on- and post-Dara compared to pre-Dara, while CD8+ T cells showed a 30% increase. CD38 expression was markedly reduced on- and post-Dara across most immune cell types, confirming the antibody's on-target effects and previously published results. On-Dara samples exhibited increased expression of cell surface markers associated with T cell activation and migration (HLA-DR and CXCR3), compared to pre-Dara samples. This was accompanied by a concomitant downregulation of inflammatory pathways, including TNFα and IL-2 gene signatures, potentially enhancing anti-tumor immune responses during treatment. When comparing patients in remission to relapsed patients, a significant upregulation of a senescence marker (CD57), and immune checkpoint molecules (ICOS, PD-1 and TIGIT) was observed in the T cell compartment, with the highest fold change (FC=1.98) on regulatory T cells in relapsed patients. Additionally, TIM-3 expression was significantly elevated in dendritic cells (FC=2.08) and monocytes (FC=1.44) in relapsed patients, suggesting a potential restraint of anti-tumor immunity. Moreover, interferon-stimulated gene (ISG) pathways were downregulated in NK cells and monocytes in remission but upregulated in relapsed patients, implying a potential pro-tumoral role in disease progression. **Conclusions:** Our comprehensive single-cell analyses unveil distinct immunomodulatory effects of Dara on the bone marrow immune microenvironment, including alterations in immune cell frequencies, activation states, and functional pathways. The observed upregulation of senescence markers, immune checkpoints, and pro-tumoral pathways in relapsed patients highlights potential resistance mechanisms and suggests avenues for therapy combination and sequencing strategies to enhance efficacy, overcome resistance, and improve patient outcomes in MM.

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Artificial Intelligence System To Predict t(11,14) Status in Multiple Myeloma Patients by Leveraging the Correlation of Plasma Cell Morphology and Genetics

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Introduction: The diagnosis of multiple myeloma (MM) is based on the cytomorphological analysis of bone marrow aspirate (BMA), along with cytometry and genetic analysis based on underlying cytogenetic alterations, which have both prognostic and therapeutic implications. An example is the translocation (t)(11;14), which, although it does not have a prognostic impact, serves as a therapeutic target by allowing targeted treatment with venetoclax in these patients. The objective of our study is to develop an artificial intelligence (AI) algorithm to automatically identify plasma cells (PC) with t(11;14) from morphological features of BMA samples analyzed using automated optical microscopy. **Methods:** We digitized 40 fields (100x) from each patient, including healthy controls, controls with MM without t(11;14), and patients with confirmed MM with t(11;14), using a device manufactured with a 3D printer to attach a mobile application to an optical microscope. For each BMA, 40 fields (100x) were digitized and the average of PC analyzed was 174. An AI algorithm was developed based on a database of more than 400,000 triple manually labeled cells, capable of automatically identifying nucleated cells from BMA images and classifying them into PC and non-PC, with an area under the curve (ROC-AUC) of 98%. Subsequently, an AI algorithm was developed to learn morphological patterns of PC associated with t(11;14) and predict its presence. The AI algorithm corresponds to a Multiple Instance Learning (MIL) architecture, which in turn uses features of each cell extracted by a foundational model trained through self-supervised learning on a database of more than 100K BMA cells. This MIL algorithm for predicting t(11;14) was trained using a 3-fold cross-validation technique, thus ensuring a division at the patient level between training and validation sets. **Results:** A total of 881 digitized cases were analyzed, 841 negative [677 healthy controls and 164 MM without t(11;14)] and 40 MM positive for t(11;14). Morphological analysis of plasma cells using the AI algorithm proved effective for detecting PCs with t(11;14) with an area under the ROC curve (ROC-AUC) of 0.88. The algorithm can predict the presence of t(11;14) with a sensitivity of 0.83 and a specificity of 0.74. The false positive rate was 0.26, while the false negative rate was 0.17. **Conclusions:** Our results demonstrate the potential of applying AI models in the morphological analysis of PC in BMA for detecting genetic alterations, confirming the morphology-genetics correlation in PC of MM cases with t(11;14). Our algorithm sensitively identifies the presence of morphological features suggestive of t(11;14); however, it shows more limited specificity, which could likely be resolved by increasing the sample size, refining the model architecture, or exploring alternative models that include other predictive variables.

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Semi-automated Interphase FISH (iFISH) Spot Scoring in CD138-Positive Cells: Validation Study for Genetic Abnormalities Detection in Multiple Myeloma

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Introduction: Genetic abnormalities detected in plasma cells are important predictors of prognosis in myeloma multiple (MM) and interphase Fluorescent in Situ Hybridization (iFISH) in selected plasma cells is the gold standard genetic technique. Manual iFISH analysis is a time-consuming, exhausting, and error-prone process that needs the participation of at least two qualified analysts. Interobserver variability can lead to misunderstandings and scoring discrepancies, while interlaboratory variability makes it difficult to compare data. Automated analysis provides an opportunity to overcome difficulties with manual analysis. Guidelines for manual fluorescent spot analysis already exist but they are not clear for automated scoring. The aim of the study was to validate the automated analysis and to compare the effectiveness of this with standard manual analysis for iFISH in selected CD138 cells. **Methods:** A workstation was optimized based on the manufacturer's configurations. Six commercial probes (CDKN2C/CKS1B, RB1/DLEU1/LAMP1, TP53/CEN17, FGFR3/IGH, CCND1/IGH, and IGH/MAF) were examined to detect gains, losses, and rearrangements of genes across a total of 180 slides (20 samples). Reference values proposed by the European Myeloma Network (10% for rearrangements and 20% for gains and losses) were used to compare the results of manual and semi-automated analyses. The time spent by the biologist for semi-automated and manual analyses was compared in another 10 samples. Statistical analyses were done using the Tau-b of Kendall coefficient (assess the reliability of the percentage of signal classes for each probe and classifier were employed to categorize the classes into normal or abnormal diagnoses), the Kappa coefficient (for accuracy) and paired Wilcoxon test (comparison the time spent for manual and semi-automated per probe). **Results:** The first important data was that automated analysis was not effective, lacked validation and was excluded. The results for the probe CDKN2C/CKS1B showed 5 cases with abnormal signals (4 gain of CKS1B), RB1/DLEU1/LAMP1 probe showed 3 cases with deletion of all probes, TP53/17cen probe detected 1 abnormal case with trisomy 17, FGFR3/IGH probe detected 5 cases with abnormal signals (1 FGFR3::IGH) MAF/IGH probe detected 5 cases with abnormal signals (1 IGH::MAF) and CCND1/IGH probe detected 7 cases with abnormal signals (4 CCND1::IGH). A 100% of accuracy for normal or abnormal signals patterns was found in manual and semi-automated analysis. Less time for analysis occurred in the semi-automated analysis than in the manual one for the CDKN2C/CKS1B probes (2.3 vs 3.9 minutes, p= 0.013) and for CCND1/IGH (2.0 vs 4.7, p=0.009). **Conclusions:** n/a.