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Next Generation Sequencing for Measurable Residual Disease Detection in Acute Myeloid Leukemia

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Karl Isaksson, Medicinaregatan 16A, Göteborg, fredagen den 29 november 2019, klockan 9:00

av **Erik Delsing Malmberg**

Fakultetsopponent: Professor Eva Hellström-Lindberg, Karolinska Institutet, Sverige.

Avhandlingen baseras på följande delarbeten:

I. **Malmberg E. B.R.**, Ståhlman S., Rehammar A., Samuelsson T., J. Alm S., Kristiansson E., Abrahamsson J., Garelius H., Pettersson L., Ehinger M., Palmqvist L. and Fogelstrand L. Patient-tailored analysis of minimal residual disease in acute myeloid leukemia using next-generation sequencing. *European Journal of Haematology 2017 Jan;98(1):26-37.*

II. **Delsing Malmberg E.**, Rehammar A., Buongermino Pereira M., Abrahamsson J., Samuelsson T., Ståhlman S., Asp J., Tierens A., Palmqvist L., Kristiansson E., and Fogelstrand L. Accurate and sensitive analysis of minimal residual disease in acute myeloid leukemia using deep sequencing of single nucleotide variations. *The Journal of Molecular Diagnostics 2019 Jan;21(1):149-162.*

III. **Delsing Malmberg E.**, J. Alm S., Nicklasson M., Lazarevic V., Ståhlman S., Samuelsson T., Lenhoff S., Asp J., Ehinger M., Palmqvist L., Brune M., and Fogelstrand L. Minimal residual disease assessed with deep sequencing of *NPM1* mutations predicts relapse after allogeneic stem cell transplant in AML. *Leukemia & Lymphoma 2019 Feb;60(2):409-417.*

IV. Løvvik Juul-Dam K.*, **Delsing Malmberg E.***, Rehammar A., Kristiansson E., Abrahamsson J., Aggerholm A., Maria Dirdal M., Jahnukainen K., Lausen B., Beier Ommen H., Hasle H.† and Fogelstrand L.† Patient-tailored deep sequencing of blood enables early detection of relapse in childhood acute myeloid leukemia. *First authors contributed equally. †Senior authors contributed equally. *Manuscript.*

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Abstract

Acute myeloid leukemia (AML) is the most common form of acute leukemia and generally associated with a poor prognosis. For both children and adults, the treatment is based on chemotherapy. Allogeneic hematopoietic stem cell transplant (alloHCT) is reserved for patients with intermediate or high risk of relapse, due to its associated risks. The initial response to treatment is a very important prognostic factor. The response is determined by the amount of residual leukemic cells in the bone marrow during treatment – measurable residual disease (MRD). The methods currently used for MRD analysis have drawbacks in terms of sensitivity and/or applicability. The work included in this thesis focused on the development, validation and investigation of the clinical applicability of a next generation sequencing based strategy for MRD analysis. The strategy was based on identification of leukemia-specific mutations, present at diagnosis and suitable for MRD, using exome sequencing. These mutations were subsequently quantified in follow-up samples using an amplicon based sequencing method, targeted deep sequencing. The study samples comprised of blood and bone marrow collected at diagnosis, during follow-up, and at relapse from adults and children with AML. As proof-of-principle, we showed in paper I that exome-sequencing could be used for identification of leukemia-specific mutations at diagnosis and that targeted deep sequencing of these mutations in follow-up samples could be used for patient-tailored MRD analysis. Paper II showed that targeted deep sequencing of single nucleotide variations (SNVs) for patient-tailored MRD analysis was accurate with good reproducibility and sensitivity meeting the consensus criterion for molecular MRD analysis (<0.1% leukemic cells). Paper III showed that measurable levels of recurrent *NPM1* insertions after alloHCT, analyzed with targeted deep sequencing were associated with higher risk of relapse and worse overall survival as compared to non-detectable levels. Paper IV showed that targeted deep sequencing of SNVs for patient-tailored MRD analysis in peripheral blood could detect increasing mutation burden before hematological relapse in children. In conclusion, the results show that targeted deep sequencing of leukemia-specific mutations is an applicable tool for MRD analysis, enabling molecular surveillance for virtually all AML patients. The method could provide better support for treatment decisions and thereby chances for improved prognosis in AML.

Keywords: Acute Myeloid Leukemia, Minimal Residual Disease, Massively Parallel Sequencing, Next Generation Sequencing, *NPM1*, alloHCT

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