

Integrating Vector Systems – Challenges to Basic, Preclinical and Clinical Development

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Integrating vector systems offer the opportunity to add therapeutic transgene information to patient's somatic stem cells, potentially for a lifetime. Our work focuses on the biology and precise localisation of retro-, lenti-, foamy- and adeono-associated virus vector integration sites in preclinical and clinical gene therapy studies. One main rationale for gene therapy in the early 1990s was the assumption that vector integration would be (semi-)random and that the chance of disrupting a gene by single integration resulting in insertional mutagenesis and/or oncogenesis would be negligible. While insertion induced oncogenesis has been reported for wild type retroviruses and related replication competent vectors, retrovirus vector based gene therapy studies were thought to lead to random monoallelic integration without considerable biological consequences due to single integration. However, clinical experience has taught us that these vectors differ less from the biology of wild type viruses than originally thought. We could previously demonstrate the tremendous influence on engraftment and proliferation of transduced cells that might result from vector integration into certain sites of the mammalian genome in preclinical and clinical severe adverse side effects. Severe side effects in a clinical gene therapy trial we described (1) have prompted current detailed RIS distribution analyses in cell lines, in human xenotransplants and non-human primates (3-5). These new data have indicated that retrovirus insertions occur preferentially in and around genes, and their location distribution is severely skewed towards the start of transcription. In 2 patients suffering X-linked SCID, vector induced upregulation of LMO2, a key regulator of early hematopoiesis, has triggered abnormal T cell proliferation, potentially enhanced by the constitutive expression of the Interleukin-2 receptor gamma chain transgene (1). The results from a third patient suffering leukemia in the same trial are still pending but presumed to involve a similar T cell oncogene. In a preclinical mouse model, we had observed retrovirus vector insertion into EVI1 as a cause of vector related leukaemia, here in putative but not proven synergy with an LNGFR transgene (2). These insertions have in common that they affect known regulators of cell type specific proliferation. These findings indicate that earlier assumptions about the choice of retrovirus insertion sites are not very well

understood, that the likelihood of untoward insertional mutagenesis and oncogenesis are largely unknown and that there is no efficient and reliable biosafety assessment of insertional side effects available. Thus, efficient biosafety assessment of integrating vector systems requires unequivocally the exact localization of the proviral DNA within the cellular genome and has prompted our studies on large-scale mapping of retrovirus integration sites using linear amplification mediated PCR (LAM-PCR) in clinical gene therapy trials.

Large-scale integration site analysis resulted in the characterization of 572 unique mappable RIS derived from engrafted T cells and leukocytes from 9 SCID-X1 patients (Alain Fischer and Marina Cavazzana-Calvo, Paris, France). RIS distribution showed no difference between the 6 healthy patients and the 3 patients who acquired a lymphoproliferative disease. We can demonstrate that integration site distribution in SCID-X1 patients is not random and that the integration occurs mainly within or close to specific regions of genes. Two-thirds of insertions occurred in or very near to genes, of which more than half are actively expressed in CD34 expressing cells, and one-third of insertions are found near (\pm 5kb) the transcription start site of gene coding regions. Strikingly, one fourth of all integrations are clustered in a small number of common integration sites, mainly near or in oncogenes and transcription factors associated with early stem cell development and cell cycling. The analysis of one patients pretransplant transduced cells (102 unique mappable RIS) showed that the percentage of CIS clones increased in post- compared to pre-transplantation samples, indicating that insertion in these gene regions has an influence on cell clone engraftment, survival and/or proliferation.

Unlike in SCID-X1 and ADA-SCID trials, where gene corrected T cells have the capability to proliferate independent of their bone marrow progenitors, gene corrected cells in myelopoiesis related inherited diseases are in need of an additional 'in vivo selection' system: CTX-R genes like MGMT. To study the hypothesis that an in vivo selective advantage of gene-corrected cells capable of long term engraftment and proliferation may also be related solely to particular vector insertion sites, we initiated a large-scale mapping of retrovirus integration sites in a successful myeloid gene therapy trial. We have retrieved more than 700 unique RIS. RIS distribution revealed preferential vector integration near or within gene coding regions and a skewing towards the transcriptional start site. Strikingly, we observed that 5 months after treatment, RIS distribution became highly non-random and that vector insertional

activation of PR domain gene triggered a 3- to 5-fold expansion of the transduced cell pool, leading to a sustained long-term transgene expression without signs of leukemogenesis.

Furthermore, we have started to test the potential of self-inactivating (SIN) long terminal repeat vectors to avoid insertional gene activation by removing the LTR promoter/enhancer sequences. Preclinical testing is currently ongoing to prove whether gene activation derived genotoxicity is reduced or altogether avoidable by this strategy.

References:

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