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FA iPS: correction or reprogramming first?

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In this issue of Blood, Müller et al show that Fanconi anemia (FA) cells are resistant, but not completely refractory, to reprogramming into induced pluripotent stem (iPS) cells. Genetic complementation before reprogramming restores DNA repair, rescues reprogramming, and yields iPS cells less likely to contain genetic alterations.

Fanconi anemia is a genetic disorder caused by loss of function of any of 14 genes in the FA pathway, a pathway coordinating cellular DNA damage repair mechanisms particularly involved in protection from DNA cross-linking agents. Clinically, FA manifests with bone marrow failure and increased propensity to malignancy. Genetic complementation of the FA pathway has been shown to correct the hematopoietic defects in mouse models and in cases of somatic mosaicism arising from spontaneous reversion of the mutations in humans (natural gene therapy). However, a major obstacle to translation of this promising alternative therapy for FA is the low yield of autologous hematopoietic stem cell (HSC) harvests from FA patients and the difficulties in their in vitro culture. Induced pluripotent stem cells offer the possibility to generate unlimited numbers of patient-specific cells and provide, from this perspective, a promising alternative therapy for FA. But pluripotent stem cell gene therapies come with their own set of problems. Reprogramming and prolonged expansion of cells may induce genomic alterations. And genetic correction should not imposes additional genotoxicity.

The authors of a previous study were successful in deriving FA patient iPS cells only after the genetic defect was corrected by lentiviral transfer of the FANCA gene in the fibroblasts before reprogramming and not from uncorrected cells, which lead to the conclusion that restoration of the FA pathway is an absolute prerequisite for reprogramming. In contrast, in the present study, Müller and co-workers show that a defective FA pathway does not completely abolish reprogramming. The authors set to further investigate the reprogramming defect of FA cells and its mechanisms. They show that murine Fanca−/− and Fancc−/− fibroblasts have a higher incidence of dsDNA breaks—both pre-existing and induced during reprogramming—and exhibit increased reprogramming-induced senescence compared with wild-type (wt) fibroblasts (see figure). They also show that reactive oxygen species generation during reprogramming contributes to chromosome breaks and that hypoxia has a more beneficial effect in boosting reprogramming of FA than wt cells, presumably by preventing oxidative damage. Genetic correction of murine Fanca−/− fibroblasts reduces senescence and restores the reprogramming efficiency to wt levels. Similarly, genetically corrected human FA cells exhibit enhanced reprogramming compared with uncorrected fibroblasts. Importantly, the present study suggests that corrected FA iPS cell lines are less likely to contain chromosomal aberrations. These findings taken together point to a model that links the reprogramming deficit to the deficient dsDNA repair, thereby decreasing the efficiency of iPS cell generation. Genetic correction restores these defects and rescues the reprogramming efficiency. iPS cells derived from a previously corrected somatic cell may be less likely to harbor genomic aberrations. Professional illustration by Debra T. Dartez.

Defective DNA damage repair in FA cells induces dsDNA breaks and promotes senescence on reprogramming, thereby decreasing the efficiency of iPS cell generation. Genetic correction restores these defects and rescues the reprogramming efficiency. iPS cells derived from a previously corrected somatic cell may be less likely to harbor genomic aberrations. Professional illustration by Debra T. Dartez.
practical or even feasible. This may be particularly true with FA patient cells, which expand poorly in culture and senesce early. On the other hand, iPS cells offer a much superior platform for sophisticated genetic engineering than any somatic cell, allowing for careful selection and characterization of corrected cells, for example, by screening for safe vector integration sites or by gene targeting by homologous recombination.\(^4\)\(^5\) Furthermore, reprogramming itself may induce DNA damage, exacerbated in a background of defective DNA damage repair, but cells that harbor a great mutational load seem to be selected against (accounting for the reduced reprogramming efficiency in FA).\(^6\)\(^7\) Finally, reprogramming of FA cells also selects for genetically corrected cells that express the therapeutic transgene, as demonstrated in both the present study and the study by Raya et al,\(^3\) so it may be thought greatest, G-CSF priming has had a Thoughtful improvement in EFS (hazard ratio [HR] 0.75, \(P = .01)\) and OS (HR 0.75, \(P = .01\)) occurring only in 72% of patients with intermediate risk cytogenetics. Despite these results, G-CSF priming has not found widespread acceptance.

To Pabst et al’s great credit a primary purpose of the current, and larger, study (HOVON-42) was to confirm the findings of AML-29, as well as to see if the OS benefit might be more widespread. HOVON-42 was initially conducted within the context of a randomization to either conventional dose ara-C, given as in AML-29, or escalated dose ara-C: cycle 1 = 1g/m\(^2\) twice daily \(\times 10\), cycle 2 = 2g/m\(^2\) twice daily days 1, 2, 4, and 6. Within each of these groups patients were randomized to + / − G-CSF, given during each cycle’s chemotherapy. Nine hundred seventeen patients were randomized to + / − G-CSF with 709 receiving conventional dose and 207 escalated dose ara-C. Despite a similar difference between the conventional-dose ara-C arms of AML-29 and HOVON-42, the latter could not reproduce the decrease in relapse risk seen generally in the G-CSF arm of the former, nor the improvement in EFS and OS observed in the intermediate-risk cytogenetic group when given G-CSF (HRs 0.95 and 1.01, respectively, in HOVON-42). There was, however, the above-noted improvement in EFS (HR 0.59, \(P = .003\)) and OS (HR 0.65, \(P = .012\)), due primarily to less risk of relapse, in the escalated dose ara-C group given G-CSF.

Pabst and colleagues explicitly seek explanations for the discrepant results, but find none specifically related to the 2 studies that appear plausible. They clearly recognize the possibility that the improved EFS and OS in patients given escalated dose ara-C + G-CSF in HOVON-42 will eventually prove to be a chance observation, even though they adjusted the above-noted \(P\) values to reflect several tests of statistical significance they performed.

Therapeutic findings aside, Pabst et al’s report is an important reminder of the limitations of even very well conducted randomized trials (phase 3) such as AML-29 and HOVON-42. There are several reasons why such trials may prove misleading. Most basically, as the authors imply, the results are statistics, not facts. Assume that among 100 new treatments for AML, 90 are truly not useful while 10 are truly useful; history suggests this is not unrealistic.\(^5\) Further assume a phase 3 trial formulated to have a 5% false positive rate (ie, \(P = .05\)) and a 20% false negative rate (ie, power = 80%). Eight of the 10 truly useful treatments will be called useful as will 4 of the truly not useful treatments. Hence,
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