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# A step closer toward therapies for p63-related disorders

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**Small molecules with low molecular weight are of interest for drug development, as they are more likely to be absorbed. In cancer research, *p53* is often mutated in many tumors, and many small molecules targeting mutant p53 have been tested. One of such low molecular weight compounds is APR246/PRIMA-1$^{MET}$ that was identified as a compound targeting and reactivating p53 mutants based on a cell-based screening for rescuing the apoptotic activity of p53. Recently, we have reported two different model systems, (1) corneal epithelial cells differentiated from induced pluripotent stem cells (iPSCs) derived from reprogramming of patient fibroblasts and (2) skin organotypic reconstitution of patient-derived keratinocytes. We have shown that APR246/PRIMA-1$^{MET}$ can rescue epithelial differentiation defects caused by mutations in *p63* that is a family member of p53 and shares high sequence and structural similarity with the p53 protein.[1,2] The rationale of the two cellular models for drug screening and the potential of APR246/PRIMA-1$^{MET}$ to restore visual impairment of patients are discussed (Fig. 1).**

## APR246/PRIMA-1$^{MET}$ Rescues EEC Patient Cell Derivates

The p53 family of transcription factors includes p53, p63 and p73. All three family members share a high homology at the protein level, especially in their DNA-binding domains. Nevertheless, these three transcription factors seem to exert diverged biological functions. Mutated in approximately 50% of human tumors, p53 plays a key role in tumor suppression,[3,4] whereas p73 and p63 have been demonstrated as regulators in development. For example, mutations in p63 lead to defects in ectodermal related structures such as skin and cornea.[5,6] Due to the high homology in the DNA-binding domain of p53 and p63, the compound APR246/PRIMA-1$^{MET}$ that re-activates p53 mutants used in cancer treatment (clinical trial phase I/II)[7] might be able to restore the activity of p63 in ectodermal development. Based this rationale, we tested APR246/PRIMA-1$^{MET}$ in primary cells established from ectrodactyly, ectodermal dysplasia and cleft lip/palate (EEC) syndrome patients carrying mutations in the p63 DNA-binding domain. EEC patients have limb malformation, orofacial clefting and ectodermal dysplasia that include defects in skin, hair, teeth, nails and several exocrine glands. Moreover, EEC patients suffer from progressive limbal stem cell deficiency (LSCD) associated with ocular surface inflammation leading to a progressive keratopathy. LSCD alters corneal transparency with dense vascularized corneal pannus, eventually leading to visual impairment. Two different model systems were tested with APR246/PRIMA-1$^{MET}$, namely corneal epithelial cells derived from patient fibroblasts that were first reprogrammed into induced pluripotent stem cells (iPSCs)[1] and keratinocytes from patient epidermis.[2] Examined by corneal-specific gene expression, iPSCs established from p63 EEC patients failed to differentiate into corneal epithelial cells, whereas treatment with APR246/PRIMA-1$^{MET}$ restores corneal epithelial commitment. Similarly, skin keratinocytes established

form p63 EEC patients that exhibit defects in epidermal differentiation were treated with APR246/PRIMA-1$^{MET}$ and expression of epidermal marker genes was enhanced. In both model systems, the restoration of p63-mediated differentiation is likely through rescue of expression of p63 target genes. The success of this rational drug testing suggests an interesting possibility of developing targeted therapy for phenotypically distinct diseases that are caused by a similar underlying molecular mechanism.

## Flexible Cellular Models for Drug Screening

The cellular models we reported are flexible in vitro systems for studying p63 pathogenesis. As skin is one of structures affected in p63 patients, keratinocytes isolated from the basal layer of the patient skin are probably the most relevant cells to study affected epidermal development in these patients. Our in vitro differentiation model of the skin keratinocytes recapitulated the epidermal differentiation process based on the marker gene expression in each sublayer of the epidermis. This model therefore can be used to study detailed cellular and molecular disease mechanisms in the skin of p63 patients. In addition to skin problems, patients with p63 mutations also often suffer limb malformation, cleft lip/palate and urinary and kidney problems.[5] In addition, p63 mouse models have also shown defects in heart and thymus development.[8,9] The proper development of these structures is dependent on proliferation and differentiation of different types of cells and tissues. Not all these cells and tissues are easily available from patients. The iPSCs from p63 patients can be differentiated into different cell lineages and therefore provide a suitable system to study the disease mechanisms for other p63 phenotypes and to perform drug screening.

## In Vivo Models for Therapies Toward EEC Syndrome

To establish that APR246/PRIMA-1$^{MET}$ can be used in treatment for EEC patients with mutations in the p63 DNA-binding domain, in vivo studies are essential. Up to now, the existing mouse models do not seem to be suitable for this purpose. The best described mouse model for p63 with clear phenotypes in skin, orofacial regions and limbs, which resemble to the human p63 disease phenotypes is the p63 knockout mouse.[10,11] However, as there is no p63 or only wild type p63 (heterozygous knockout) present in these mice, the effect of APR246/PRIMA-1$^{MET}$ cannot be envisaged. Although an R279H knock-in mouse model has been reported for EEC syndrome,[12] except the limb phenotype in the homozygous knockout mice, the detailed description of other phenotypes are yet to be described. A faithful mouse model is necessary to test the effect of APR246/PRIMA-1$^{MET}$ on developmental processes that are controlled by p63. Furthermore, there is a clear clinical variability among EEC syndrome patients with p63 mutations,[13] which suggests that individual genetic background is an important factor that contributes to the phenotypes, and an animal model might not be sufficient to address the efficacy of APR246/PRIMA-1$^{MET}$. This assumption is in line with our observations of the differential effects of APR246/PRIMA-1$^{MET}$ toward different p63 mutations.[1,2] Therefore, it is desirable to test APR246/PRIMA-1$^{MET}$ in in vivo models with transplants or grafts established from patient cells.

## Cornea as an Ideal Target for EEC Therapy

Although there is no suitable animal model for EEC syndrome, there are several arguments that APR246/PRIMA-1$^{MET}$ treatment can directly be evaluated on the cornea in humans. First, the cornea is an ideal tissue for therapy due to its ease of access. Second, the cornea epithelium is avascular in its final form, creating a relative privileged site suitable for transplantation. Third, successful restoration of vision in human patients is easy to monitor. Fourth, the LSCD-related visual impairment of EEC patients appears at advancing age, allowing treating patients before or at the genesis of the symptom. Fifth, APR246/PRIMA-1$^{MET}$ drug has been already tested in phase I/II clinical trials on patients suffering from refractory hematologic malignancies and prostate cancer.[7] The drug was administrated intravenously without adverse effects. Therefore, these clinical data strongly suggest that administration of the drug locally as eye drops should be as safe as possible. Surgical removal of the keratinized corneal epithelium of EEC patients will induce a wound healing and activation of mutated p63 gene expression.[14] APR246/PRIMA-1$^{MET}$ drug should restore normal function of p63 and its target genes. Nevertheless, as an alternative to animal model, EEC-iPSC could be injected onto wounded mouse cornea in absence and presence of APR246/PRIMA-1$^{MET}$ to evaluate in vivo the therapeutic effect of the drug.

## APR246/PRIMA-1$^{MET}$-*Responsive Mutants*

Consistently in iPSC and keratinocytes models, individual p63 mutations, R304W and R204W, which both are the DNA-binding domain of p63, showed different response to APR246/PRIMA-1$^{MET}$ treatment. The R304W mutant is more amenable to re-activation by APR246/PRIMA-1$^{MET}$ than the R204W mutant, which might be due to the molecular nature of the two mutations. It has been shown that APR246/PRIMA-1$^{MET}$ treatment leads to different response toward p53 mutants.[15] R304 in p63 corresponds to R273 in p53, a residue that makes direct contact with DNA. Mutations of R273 in p53 retains wild type conformation to a large extent.[16] In contrast, R204 in p63 corresponds to p53 R175 that is important for the structural integrity of the DNA-binding domain, and mutations of this amino acid show severe structural defects. The different response of p63 mutants to APR246/PRIMA-1$^{MET}$ treatment is in agreement with previously shown effects of APR246/PRIMA-1$^{MET}$ on the TAp63γ isoform that APR246/PRIMA-1$^{MET}$ restored pro-apoptotic property of TAp63γR304W while it induced mainly growth arrest in cells with TAp63γR204W.[17] It will be of interest to test other DNA binding mutations in p63 that are involved in EEC syndrome.

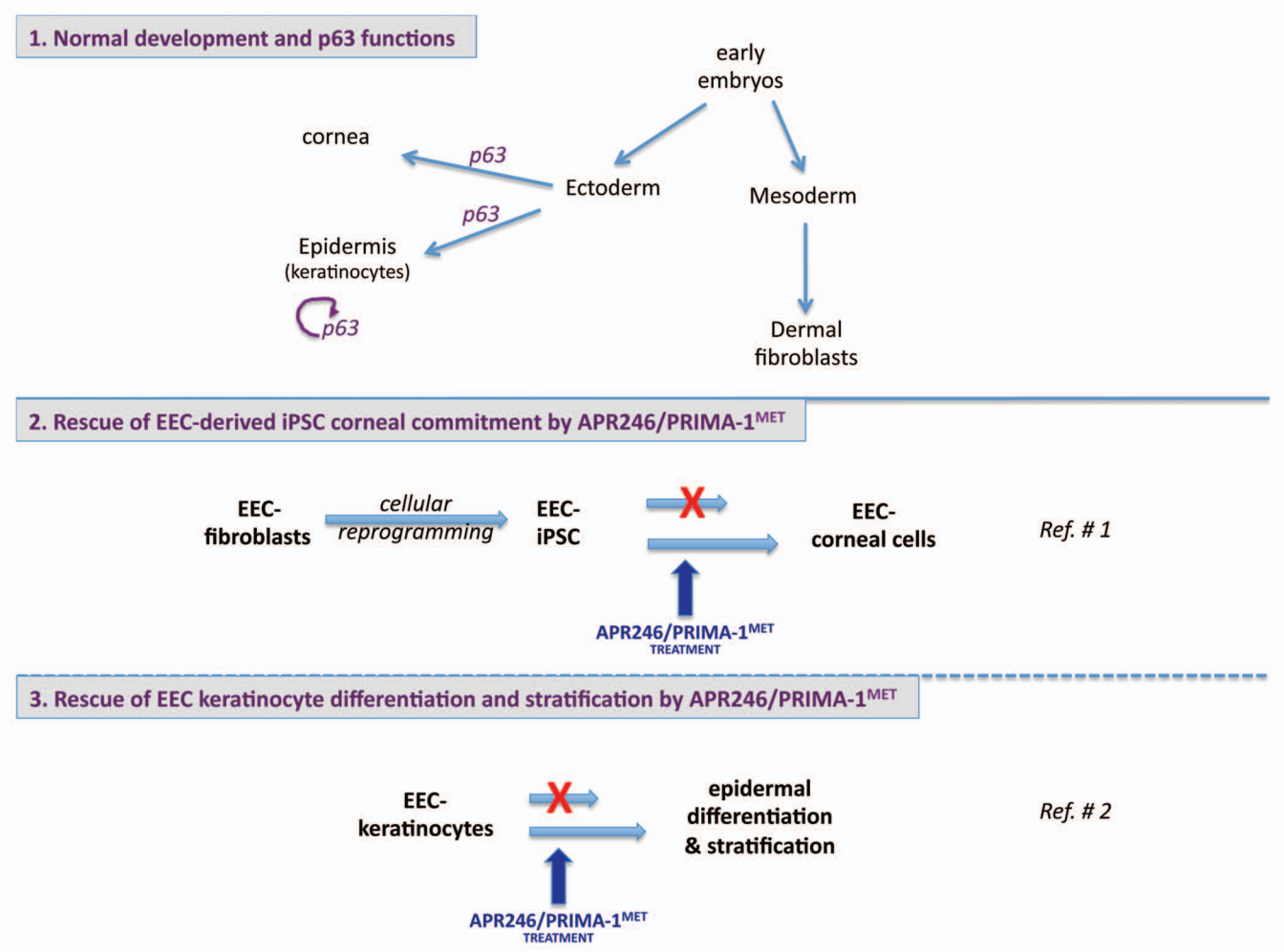


**Figure 1.** Description of the two cellular models used to demonstrate the effect of the small compound APR246/PRIMA-1$^{MET}$ on epithelial commitment and differentiation. (**1**) p63 is required for proper epidermal and corneal commitment of ectodermal progenitors during mammalian development[19] and epidermal stem cells/progenitors self-renewal and differentiation.[20] (**2**) Dermal fibroblasts (or skin keratinocytes) can be reprogrammed into embryonic-like cells called induced pluripotent stem cells (iPSC) in vitro.[21] EEC-iPSC derived from reprogramming of fibroblasts are unable to differentiate into corneal epithelial cells properly and treatment with APR246/PRIMA-1$^{MET}$ improved corneal commitment. (**3**) Keratinocytes isolated from EEC patients defects in epidermal differentiation and stratification, which are partially restored with APR246/PRIMA-1$^{MET}$ treatment.

## Alternative Universal Drugs

The choice of APR246/PRIMA-1$^{MET}$ is based on our rational interpretation of the molecular mechanism of mutations in p53 in cancer and in p63 in development. A number of compounds have been developed toward p53 mutations. Among them, p53R3 that specifically restores DNA binding of p53 mutants R175H and R273H[18] will be of great interest to test. Other compounds that can stabilize p53 mutants through other mechanisms such as PhiKan083 and CP-31398[15] could also be tested for p63-related disease models. Furthermore, our cellular systems can also be used or further optimized for screening of a panel of compounds, including those are already approved for clinically use, to improve the phenotypic defects of p63 patients.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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