REVIEW ARTICLE

The Promise of Genomics and the Development of Targeted Therapies for Cutaneous Squamous Cell Carcinoma

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Targeted therapies for melanoma and basal cell carcinoma have evolved from deciphering the molecular mechanisms involved in their tumourigenesis. Mutations in *BRAF* have led to clinical use of BRAF-inhibitors in advanced melanoma, and mutations in Hedgehog signaling to smoothened inhibitors in basal cell carcinoma. The development of tumour resistance to these treatments is leading to many new drug development initiatives and the exploration of multiple signalling pathways. Cutaneous squamous cell carcinoma continues to rise steeply in incidence with very limited therapeutic options for locally advanced or metastatic disease. New genetic technologies find significant levels of mutation in Notch gene family as well as other already recognized gene mutations, such as *TP53*. The mutational burden in cutaneous squamous cell carcinoma is massive, challenging the identification of driver genes and inhibiting translation from genomics to the clinic. Clinical experience with targeted therapies, such as epidermal growth factor receptor inhibitors, or immune modulatory drugs suggests that these agents may be of benefit to patients, while a more complete understanding of the mechanisms behind squamous cell carcinogenesis awaits further progress. *Key words*: *Cutaneous squamous cell carcinoma (cSCC); gene mutation; targeted treatment.*

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Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer (NMSC) globally, with an increasing incidence, which accounts for one million new cases diagnosed annually in the US (1). The risk of further malignancies after a cSCC is significantly increased and the associated morbidity, mortality and costs to healthcare providers are substantial. Most cSCC are likely to arise from actinic keratoses (AK) or full thickness carcinoma-*in-situ* (CIS, Bowen's disease), and are readily treated and cured with surgery or radiation. However, a small subset of 'high risk' cSCC – approximately 5–10% – display more aggressive behaviour, and outcome is particularly poor for metastatic disease, occurring most commonly in the elderly, dependant on the extent of nodal involvement with recurrence occurring most often in the first 2 years (2). Treatment options currently available for locally advanced or metastatic disease have limited effects on overall survival and new systemic treatments are badly needed (3, 4). Current staging systems have identifed high-risk features as including tumour size (maximum horizontal diameter > 2 cm), tumour depth of invasion (> 2 mm or Clark Level > IV), anatomic location (ear and lip), perineural invasion, and poor differentiation (5). Morbidity and mortality of cSCC is a relatively unrecognised public health issue – 'an under-estimated nemesis' (6). The health burden of cSCC is substantial, indeed, in some areas of the USA it has been estimated that cSCC may have caused as many total deaths as melanoma in 2012 (7).

The promise of new genomics capabilities in directing development of targeted anticancer agents is showing early benefit for melanoma, basal cell carcinoma and dermatofibrosarcoma protruberans. In comparison, attempts to identify molecular mechanisms and pathways underlying cSCC carcinogenesis have proved less successful in delivering therapeutic targets. The advent of massively parallel (so-called 'next generation') sequencing techniques is both facilitating and complicating this field by demonstrating a very high burden of mutations in cSCC, which makes determination of driver genes daunting, particularly in terms of distinguishing them from passenger mutations that may have minimal impact on tumour progression. Nonetheless, an emerging analysis of the cSCC oncogenome is that, although the alterations observed in individual tumours may be distinct, they largely participate in a network of dysregulated molecular pathways that appear to be shared by the majority of cSCC (8). This review considers current progress in understanding the cellular and molecular basis of cutaneous squamous cell carcinogenesis and its potential implications for future development of personalized cSCC therapies.

AETIOLOGY OF CUTANEOUS SQUAMOUS CELL CARCINOMA

The 3 major groups of environmental or host-dependent risk factors identified for cSCC are ultraviolet radiation (UVR) exposure, genetic predisposition and immunosuppression (Fig. 1).
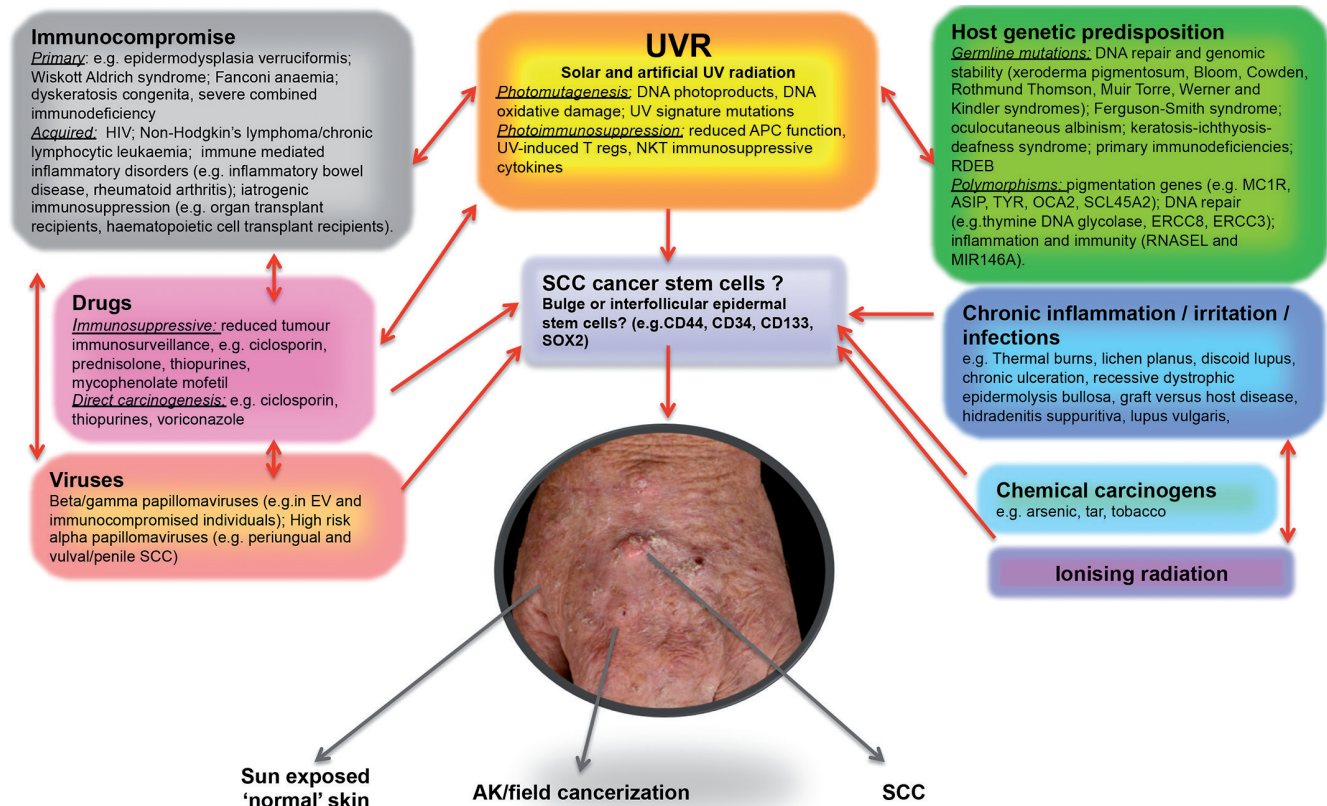
Immunocompromise
Primary: e.g. epidermodysplasia verruciformis; Wiskott Aldrich syndrome; Fanconi anaemia; dyskeratosis congenita, severe combined immunodeficiency
Acquired: HIV; Non-Hodgkin's lymphoma/chronic lymphocytic leukaemia; immune mediated inflammatory disorders (e.g. inflammatory bowel disease, rheumatoid arthritis); iatrogenic immunosuppression (e.g. organ transplant recipients, haematopoietic cell transplant recipients).

UVR
Solar and artificial UV radiation
Photomutagenesis: DNA photoproducts, DNA oxidative damage; UV signature mutations
Photoimmunosuppression: reduced APC function, UV-induced T regs, NKT immunosuppressive cytokines

Host genetic predisposition
Germline mutations: DNA repair and genomic stability (xeroderma pigmentosum, Bloom, Cowden, Rothmund Thomson, Muir Torre, Werner and Kindler syndromes); Ferguson-Smith syndrome; oculocutaneous albinism; keratosis-ichthyosis-deafness syndrome; primary immunodeficiencies; RDEB
Polymorphisms: pigmentation genes (e.g. MC1R, ASIP, TYR, OCA2, SCL45A2); DNA repair (e.g.thymine DNA glycolase, ERCC8, ERCC3); inflammation and immunity (RNASEL and MIR146A).

Drugs
Immunosuppressive: reduced tumour immunosurveillance, e.g. ciclosporin, prednisolone, thiopurines, mycophenolate mofetil
Direct carcinogenesis: e.g. ciclosporin, thiopurines, voriconazole

SCC cancer stem cells ?
Bulge or interfollicular epidermal stem cells? (e.g.CD44, CD34, CD133, SOX2)

Chronic inflammation / irritation / infections
e.g. Thermal burns, lichen planus, discoid lupus, chronic ulceration, recessive dystrophic epidermolysis bullosa, graft versus host disease, hidradenitis suppuritiva, lupus vulgaris,

Viruses
Beta/gamma papillomaviruses (e.g.in EV and immunocompromised individuals); High risk alpha papillomaviruses (e.g. periungual and vulval/penile SCC)

Chemical carcinogens
e.g. arsenic, tar, tobacco

Ionising radiation

Sun exposed 'normal' skin   **AK/field cancerization**   **SCC**

Fig. 1. Summary of aetiology factors in cutaneous squamous cell carcinoma.

UVR has been classified as a class I carcinogen by IARC (International Agency for Research on Cancer), sufficient for initiation, promotion and progression of squamous carcinogenesis. It is the most important environmental risk factor for cSCC, causing DNA damage that leads to aberrations in oncogenes and tumour suppressor genes and induction of immunological tolerance. UVR-induced mutagenesis results in characteristic C-T and CC-TT dipyrimidine transitions, which constitute the majority of mutations found in cSCC. Individuals who have had high cumulative UV irradiation, especially if fair skinned and easily sunburned, are at particularly increased risk.

Genetic predisposition to cSCC is well recognised in certain family cancer syndromes. Patients with inherited defects in DNA repair and genomic stability are at significantly increased risk of cSCC and include those with aberrations in nucleotide excision repair genes *XPA-G* and *XP-V* (xeroderma pigmentosum, XP), *BLM* (Bloom syndrome), *PTEN* (Cowden syndrome), FANCA-N (Fanconi Anemia), *TP53* (Li-Fraumeni syndrome), *RECQL4* (Rothmund-Thomson syndrome), *WRN* (Werner syndrome), telomere maintenance (dyskeratosis congenita), *FERMT1* (Kindler syndrome) and mammalian mismatch repair (Muir Torre syndrome). Mutations in genes involved in melanin synthesis (*TYR*, *TRYP1*, P-protein and *MATP*) underlie oculocutaneous albinism. Patients with recessive dystrophic epidermolysis bullosa (RDEB), an hereditary deficiency of type

VII collagen due to mutations in *COL7A1*, develop aggressive cSCC (9). Ferguson-Smith syndrome, also known as multiple self-healing squamous epitheliomata (MSSE) and characterized by spontaneous resolution of cSCC, is due to germ line mutations of *TGFBR1* (10).

Host specific genetic changes predisposing to cSCC have been investigated in genome-wide association studies (GWAS) and locus-specific studies of germline single nucleotide polymorphisms (SNPs). These studies have particularly focused on genes involved in skin pigmentation and DNA repair. Clinical skin phenotype is defined by constitutive and facultative pigmentation, which are controlled by more than 150 genes. A recent systematic review has highlighted correlations between SNPs and SCC risk in pigment genes, which were retained after controlling for clinical skin phenotype traits (11). Melanocortin-1 receptor (*MC1R*) controls facultative pigmentation, stimulated by UV-induced alpha-MSH production in keratinocytes; *MC1R* red hair colour variants Arg151Cys and Arg160Trp and the alpha-MSH antagonist, Agouti signaling peptide (*ASIP*) AH haplotype, were the most highly correlated SNPs. Roles for these genes other than in control of pigmentation may be important and include immune and inflammatory responses to UV radiation, proliferation and differentiation.

Given the greatly increased incidence of cSCC in inherited DNA repair disorders such as xeroderma pigmentosum, SNPs in DNA repair genes have been exa-

mined and there is some evidence that common variants may increase cSCC risk. In one large, population-based study of NMSC risk (BCC and cSCC), SNPs in the base excision repair gene, thymine DNA glycosylase, were most strongly associated. Significant associations also identified variants of genes involved in nucleotide excision repair (*ERCC8, ERCC3*), homologous recombinational repair (*PALB2, DMC1*), direct reversal repair (*MGMT*), DNA damage signal transduction (*CHEK2*) and mismatch repair (*MSH6*) (12). In immunosuppressed organ transplant recipients (OTRs), associations have been identified between cSCC and p53 72-R homozygosity (13) with additional associations also including polymorphisms in the detoxifying enzyme glutathione-S-transferase, IL-10, *COX2* gene regulatory region variants, folate pathway-related methylenetetrahydrofolate reductase (*MTHFR: C677T*) polymorphisms and vitamin D receptor (Intron8G/T) (14).

Innate or acquired immunosuppression is a significant risk factor for cSCC. Prolonged iatrogenic immunosuppression in OTRs results in up to 150-fold increased risk of cSCC, with a reversal of the usual BCC:SCC ratio (15) and iatrogenically immunosuppressed individuals such as those with immune mediated inflammatory disorders, e.g. inflammatory bowel disease and rheumatoid arthritis, are also at risk, as are patients with disease-induced immunosuppression, such as human immunodeficiency virus infection and chronic lymphocytic leukaemia. The mechanisms responsible are likely to be multifactorial: in addition to reduced tumour immunosurveillance, several of the immunosuppressive drugs are known to have direct carcinogenic effects, often in combination with UVR. A putative role for viruses, in particular beta-papillomaviruses, may be especially significant in the context of immunosuppression, but is beyond the scope of this review.

GENETIC AND EPIGENETIC ALTERATIONS IN CUTANEOUS SQUAMOUS CELL CARCINOMA

The transformation of a normal keratinocyte into a premalignant AK, CIS (Bowen's disease), invasive cSCC and metastasis is likely to be a multistep process requiring the accumulation of somatic genetic and epigenetic alterations in key signaling pathways regulating cell fate, cell survival and genome maintenance (16) (Table I). The concept of 'field cancerization' was introduced by Slaughter in 1953 to describe the observation that multiple primary head and neck SCC (HNSCC) arise within close proximity, suggestive of a pre-disposed area of tissue, or 'field', and this is now widely accepted in sun-exposed skin characterized by extensive clones of p53 mutation (17). The most prominent genetic changes identified to date at all stages of cSCC and how they may influence development of future personalized treatment for cSCC are summarized below.

Chromosomal alterations in cSCC

Changes in chromosomal number, deletions, insertions and translocations are well recognized in many cancers. cSCC karyotypes are particularly complex and display large numbers of allelic imbalances (18). Comparative genomic hybridization (CGH) and multiplex fluorescence *in situ* hybridization studies have confirmed this complexity, with frequent loss of 3p, 9p and gain of 11q (19). Structural aberrations of centromeric regions have been found by CGH with consequent whole-arm translocations and duplication of chromosome arms causing formation of iso-chromosomes or copy number-neutral loss of heterozygosity (LOH) particularly involving 3q, 8q and 9q. LOH studies using microsatellite PCR techniques and, more recently, genome-wide SNP arrays, have confirmed recurrent regions of loss and gain in cSCC: loss of 3p (65%), 9p (75%), 2q, 8p and chromosome 13 and gains on 3q, 8q, 9q and 11q are consistently recognised but substantial genomic instability is also seen in AK, with alterations at 3p, 9p, 13q, 17p and 17q. SNP array studies suggest that the extent of genomic instability correlates with differentiation status, significantly fewer changes being identified in well-compared with poorly-differentiated cSCC, though not correlating with immune status (18). SNP array studies have also identified a common microdeletion at 9p23 within the locus of the gene encoding protein tyrosine phosphatase delta (*PTPRD*), which was significantly associated with a risk of metastatic progression and has also been found in glioblastoma, lung cancer and head and neck cancer (20, 21). However, a 'driver' role for PTPRD remains unproven.

Telomere dysfunction appears to be associated with chromosomal instability in cSCC and may be an early event (19). Telomeres, the hexanucleotide TTAGGG repeats at chromosome ends, are important in maintenance of chromosomal stability; erosion and formation of critically short telomeres results in chromosomal fusion and eventually breakage. Recent evidence suggests that there are two distinct cSCC subtypes – those with short/intermediate homogeneous telomeres and those with longer/heterogeneous telomeres; the former are associated with significantly greater p53 expression and karyotypic complexity (22). Telomere aggregate formation is also associated with chromosomal instability in cSCC and contributes to the multi-chromosomal translocations observed in cSCC.

Specific genetic changes in human cSCC

It has been proposed that most cancers contain 2–8 somatic 'driver' gene mutations that confer selective growth advantage, tumour initiation coinciding with the appearance of the first driver mutation (23). The majority of mutations are regarded as 'passengers' (24) – they confer no growth advantage and may even arise before tumour initiation in self-renewing tissues such

as skin. Whole exome sequencing (WES) is highlighting the very high burden of mutation in cSCC, which is significantly greater than in other solid tumours such as lung, breast or colon, with an average of one mutation per 30,000 base pairs of coding sequence (8). The extensive genomic aberrations in cSCC have hampered identification of critical drivers and it is becoming increasingly apparent that multiple genes and pathways are likely to be involved. Published research has particularly focused on *TP53, NOTCH, RAS, EGFR, SRC-family kinase (SFK), CDKN2A, NF-KB, TGFß* and, most recently, *KNSTRN*.

TP53 mutations occur early in human cSCC. It is well established that mutations in the *TP53* gene have an important and early role in the pathogenesis of cSCC; they are usually UV signature mutations and occur in up to 90% of all cSCC (25). *TP53* exerts its critical tumour suppressor function through mechanisms including induction of apoptosis, cell cycle arrest and senescence and plays a fundamental role in the response to UV damage.

Clones of mutated *TP53* are present in keratinocytes of sun-exposed normal skin (26) and are related to age and lifetime cumulative UV exposure, suggesting extensive tolerance of keratinocytes to UV-induced genetic damage (17). Their expansion appears to be driven by ongoing UVB exposure, which preferentially spares apoptosis-resistant *TP53* mutant cells, allowing accumulation of further genetic damage and uncontrolled proliferation of keratinocytes (27). *TP53* mutation is also highly prevalent in AK and, where temporal evolution of *TP53* mutation acquisition can be plotted, loss of the second p53 allele appears to be a critical event in cSCC development; expansion of mutations and development of chromosomal aberrations appear to follow complete loss of p53 function by biallelic inactivation (28).

NOTCH genes are highly mutated in human cSCC. Increasing evidence supports of a role for aberrant Notch signaling in cSCC (8, 29). The Notch family of genes encodes 4 trans-membrane receptors: *NOTCH1* is a direct transcriptional target of p53 and in the skin

Table I. *Molecular changes reported in the progression of actinic keratoses (AK) to cutaneous squamous cell carcinoma (cSCC)*

Molecular changes	Sun-exposed 'normal' skin	AK/field cancerization	cSCC	Metastasis
Cytogenetic changes		LOH: 3p, 9p, 13q, 17p, 17q	LOH: more extensive than AK (9p loss 75% and 3p loss 65%; recurrent losses 2q, 8p, 13; allelic gain on 3q, 8q and 11q) CGH: loss of 3p, 9; gain of 11q; isochromosomes 3q, 8q and 9q M-FISH: loss of 3p, 5q, 8p,17p; gain of 3q, 5p 7p, 8q and 11q. SNP: fewer changes in WD compared with MD and PD Telomeres: telomere aggregates; short/homogeneous versus long/heterogeneous	Microdeletion at 9p23
Gene mutation/ dysregulated gene/ expression/signalling	*NOTCH* mutation *TP53* mutation *FAT1* mutation *FGFR3* mutation *RBM10* mutation *RAS* mutation	*TP53* mutation *NOTCH1/2* mutation *CDKN2A* mutation *KNSTRN* mutation *RAS* mutation EGFR NFκB SFKs and Srcasm	*TP53* mutation 40–90% *NOTCH1/2* mutations in >75% *CDKN2A* mutation or methylation *TGFBR1* mutation *RAS* mutation *KNSTRN* mutation EGFR MAPK-PI3K-AKT-mTOR SFKs and Srcasm NFκB FOXM1 *C-MYC* amplifications PTEN Mir21, 124, 125b, 203, 214, 361-5-b, 365 TINCR	EGFR miRNA9 PTPRD FRZB methylation
Tumour microenvironment		NOTCH methylation in dermal fibroblasts -activation of CAFs CD8+ cells reduced	CAFs: permissive ECM, FGFs etc Immune cells: immunoregulatory cytokines (TGFβ, IL-10, VEGFA,TNFα), VEGF-C, chemokines/receptors, growth factors, MMPs E-cadherin and type VII collagen downregulation; a6b4 integrin/laminin322 over-expression MMP7 overexpression Immune response: downregulation antigen processing; reduced expression co-stimulatory receptors CD40; over-expression co-inhibitory receptors CTLA-4 and PD-1; aberrant Fas and Fas ligand pro-apoptotic proteins; COX2 overexpression OTRs: Reduced CD4^{+}, CD8^{+} and CD123^{+} cells; increased T-regs; increased IL22	E-cadherin VEGF-C

LOH: loss of heterozygosity; CGH: genomic hybridization; EGFR: epidermal growth factor receptor; CAFs: cancer associated fibroblasts; MMP: matrix metalloproteinases; OTRs: organ transplant recipients; WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated.

is expressed throughout the epidermis with Notch2 expression localized to the basal layer. Notch signaling is an important developmental pathway involved in stem cell maintenance and cell fate determination. *NOTCH1* mutations are oncogenic in haematological malignancies (including acute T-cell lymphoblastic leukaemia/lymphoma and B-cell malignancies such as chronic lymphatic leukaemia and mantle cell lymphoma). However, mutations in solid tumours tend to be loss-of-function, suggestive of a tumour suppressor role (30): 20% of HNSCC harbour mutations in *NOTCH1*, *2* or *3* (31) and WES of cSCC identified a high proportion of truncating mutations in *NOTCH1* and 2 with an overall non-synonymous mutation prevalence of 75% (29). A recent larger study of WES in 20 cSCC and targeted deep sequencing in 102 tumours reported a *NOTCH1* or *2* mutational frequency of 84%, with loss-of-function mutations often present in multiple Notch receptors within the same tumour (8). A role for Notch in cSCC development may be mediated by a number of mechanisms including p21-induced cell cycle withdrawal (32), interferon regulatory factor 6 (IRF6) (33) and p63-mediated pro-differentiation effects (34).

RAS genes are infrequently mutated, but MAPK and PI3-AKT-mTOR intracellular signalling pathways are frequently upregulated in human cSCC. The proto-oncogene RAS has been implicated in the initiation of cSCC in murine chemical carcinogenesis models (35). In human keratinocytes, experimental expression of RAS leads to growth inhibition or senescence, mediated in part by downregulation of CDK4; overexpression of CDK4 or blockade of NFκB by IKkBa overcomes this oncogenic Ras-induced cell cycle arrest and transforms primary keratinocytes into invasive cSCC (36, 37). Although in many human tumours activating mutations in *HRAS, KRAS* and *NRAS* (predominantly in codons 12,13,61) are frequent, studies of human cSCC have shown variable RAS mutation rates and indicate that the majority of sporadic cSCC do not carry detectable mutations. Recent WES and Sanger sequencing studies have confirmed this, with activating mutations of RAS – most frequently *HRAS* – in 12–20% of cSCC (8, 28).

More recently, the use of targeted BRAF inhibition in melanoma has led to further insights into the role of the RAS in cSCC. Almost 25% of patients receiving vemurafenib rapidly develop squamo-proliferative lesions, including well-differentiated cSCC, which have an increased frequency of activating RAS mutations (35–60%) compared to sporadic cSCC (8, 38). RAF is downstream of RAS and this supports the concept that there is paradoxical upregulation of MAPK signaling in *BRAF* wild-type keratinocytes exposed to BRAF inhibitors and raises the possibility that *RAS* mutations may occur in sun-exposed skin but give rise to cSCC only when combined with changes such as Notch deficiency.

Despite the increase in *RAS* mutations observed in BRAF inhibitor-associated cSCC, the frequency of *RAS* mutations in most sporadic human cSCC is low. However, an increase in levels of RAS with active GTP and upregulation of the downstream MAPK and PI3K-AKT-mTOR intracellular signaling pathways has been observed in many human cSCC (37, 39). A recent analysis of 30 laser capture-microdissected cSCCs and 10 AKs showed 196 differentially expressed genes which were enriched for processes including epidermal differentiation, migration and cell cycle regulation and metabolism, with particular involvement of MAPK signaling (40). A major challenge in interpreting transcriptome data has been a lack of consensus between studies. Reasons for this variability include the relatively small size of most sample sets, the variation in tumour characteristics and samples (e.g. the inconsistent use of microdissection and thus tumour purity and the use of frozen versus paraffin-embedded samples) and variation in the characteristics of the microarray platform used and subsequent bioinformatic analysis. Nonetheless, in cSCC, it is likely that the MAPK pathway is activated by mutation or overexpression upstream of RAS in non-*RAS* mutant tumours and/or from aberrant expression of epidermal growth factor receptor (EGFR) and/or its ligands. The PI3K/AKT/mTOR pathway appears to be the most altered mitogenic signaling pathway in HNSCC and represents an important therapeutic target (39, 40). In contrast to HNSCC, oncogenic hotspot loci mutations in *PI3KCA* and *AKT1* genes causing constitutive activation of the pathway have not been reported, although AKT2 upregulation has been observed. It is plausible that, as with MAPK, activation of the PI3K/AKT signalling in non-RAS mutant cSCC may also result from alternative mechanisms such as aberrant EGFR expression or inactivation of PTEN (phosphatase and tensin homologue deleted on chromosome 10). This important tumour suppressor is an inhibitor of the PI3K/AKT pathway and is associated with genetic predisposition to cSCC in mouse models and in Cowden's disease; it is downregulated by UVR and is required for XPC-associated global genome DNA repair after UVB exposure. Although its loss of function appears to be important in cSCC, the mechanisms responsible are not yet understood and do not appear to be due to mutation (41).

Epidermal growth factor receptor is frequently overexpressed in cSCC. In keratinocytes, EGFR (HER-1) is a member of the ErbB or HER family of cell-surface receptor tyrosine kinases (RTKs). EGFR signalling is one of the most intensely studied determinants of epithelial cell proliferation and is persistently activated in cSCC (42). In the proliferative compartment of the epidermis, EGFR signalling serves to maintain keratinocyte self-renewal and suppress differentiation, whereas in the upper layers it is downregulated. Acti-

vation in keratinocytes by binding of common ligands (such as EGF, heparin-binding EGF, TGFα, epiregulin, amphiregulin, betacellulin) or UVR leads to receptor homodimerization or heterodimerization (with another HER family member such as HER-2 and HER-3 or non-HER receptor such as c-MET and IGFR-1), activation of intracellular tyrosine kinases by autophosphorylation of tyrosine residues and phosphorylation and activation of multiple downstream pathways including PI3K-AKT-mTOR, PI3K-JAK-STAT, RAS-RAF-MEK-ERK-MAPK, PLCγ-PKC and NF-κB, resulting in increased proliferation, migration, survival, resistance to apoptosis and altered differentiation. EGFR signaling also suppresses differentiation through p53-dependent negative regulation of Notch1 gene transcription and function in keratinocytes and cSCC (43).

Dysregulated EGFR signaling can lead to neoplastic transformation and is frequently found in various forms of human cancer. This may result from a number of different mechanisms. *EGFR* activating mutations in either the extracellular or intracellular domains (insertions, deletions and missense point mutations) found in lung cancer and glioblastoma are uncommon in cSCC, and levels of reported amplifications causing overexpression are variable, ranging from 1.1–78% (44). However, EGFR can be oncogenically activated by mechanisms other than mutation and amplification, including autocrine ligand production, heterodimerisation with other EGFR family members such as ErbB2, cross-talk with heterologous receptor systems (such as integrins, G-protein coupled receptors and E-cadherin) and defective receptor downregulation (45). It has been suggested that a high proportion of primary cSCC that metastasize overexpress EGFR although overexpression does not appear to correlate with differentiation status, nor is it necessarily maintained in metastases. In HNSCC, EGFR overexpression suggestive of constitutive activation of the pathway is a common early event, which suggests a significantly dysregulated EGFR pathway in cSCC.

Src family kinase (SFK) signaling is upregulated in cSCC. SFKs are non-receptor tyrosine kinases that transduce signals from integrins and growth factor receptors and increased SFK activity is common in human cancers, including cSCC and HNSCC (46). Mutant *RAS* induces upregulation of the SFK, Fyn, which is associated with epithelial-mesenchymal transition (EMT), decreased cell–cell adhesion and increased migratory potential *in vitro*. Fyn transgenic mice spontaneously develop lesions resembling AKs and cSCCs, a process inhibited by the SFK negative regulator, Src-activating and signaling molecule (Srcasm) and elevated Fyn and decreased Srcasm levels are found in human AK and cSCC (47).

CDKN2A tumour suppressor gene inactivation is common in cSCC. Chromosome 9p loss is frequently identified in cSCC (18, 20). The *CDKN2A* locus on 9p21 encodes *p16INK4a* and *p14ARF* tumour suppressor genes and progression of AK to cSCC has been hypothesized to correlate with deletion of *p16INK4a*. Mutational frequencies of up to 50% have been identified in cSCC although epigenetic events such as methylation are also frequent as genetic mechanisms for inactivation (48).

NFκB signaling is upregulated and has pro- and anti-tumourigenic effects in cSCC. NFκB is a master regulator of epidermal homeostasis with multiple family members (RelA/p65, p50, p52, RelB and c-Rel) together with a number of upstream regulators including IKBα proteins and its effects are highly cell context dependent (49). The role of NFκB in cSCC is controversial, with some studies suggesting a pro-tumourigenic role and others a role in tumour prevention. Both inhibition and activation in keratinocytes can drive epidermal inflammation and enhanced NFκB activity appears to increase susceptibility to chemical carcinogenesis in mice (50). In human cSCC, genome-wide expression studies have shown that genes controlled by NFκB are upregulated in cSCC and AK (21). Recent studies have also shown a significant number of mutations in members of the signalling pathway such as *CARD11* (51, 52).

Transforming growth factor beta (TGFβ) receptor mutations are common in cSCC. The familial syndrome of multiple self-healing epitheliomata (MSSE), also known as Ferguson Smith syndrome, is caused by loss of function mutations in the TGFβ receptor 1 (*TGFBR1*) on 9q21 (10). Analysis of 98 human sporadic cSCC samples and 21 cSCC cell lines has also revealed mutation of TGFβ receptors in 43% of samples (Inman G, et al. Submitted for publication). TGFβ is a pleiotropic cytokine complex with often apparently contradictory effects on keratinocyte growth depending on dose and context. It is secreted complexed to latent TGFβ binding protein and latency-associated peptide and subsequently activated by proteases and/or integrins. TGFβ signals via activation of a hetero-tetrameric receptor complex of TGFβR2: TGFβR1, which results in TGFβR1 kinase driven c-terminal phosphorylation of Smad2 and Smad3. Recent clinical trial findings evaluating safety of a TGFβ blocking antibody confirm a tumour suppressive role for TGFβ signaling in cSCC (53). Although it acts as a potent tumour suppressor in the majority of cSCC, in advanced HNSCC it switches – possibly under epigenetic control – to become a potent tumour promoter (54). In addition to proliferation, other tumour-promoting activities include effects on cell survival, motility, invasion and maintenance of cancer stem cells. Few genes have more influence on the tumour microenvironment and TGFβ orchestrates epithelial-mesenchymal transition (EMT), which is central to tumour invasiveness and aggressive behaviour (54). TGFβ appears to play a role in promoting tumour heterogeneity and drug resistance in cSCC

(55). Although a pro-tumourigenic role has not yet been established in cSCC, further research is essential before TGFβ inhibitors might be used in this context in humans.

Kinestrin mutations are found in some cSCC. Recent NGS analysis of cSCC has suggested that the gene *KNSTRN* may be a previously unrecognized oncogene in human cancer (56). WES of 12 cSCC-normal pairs followed by targeted sequencing in 100 cSCC and 5 cSCC cell lines revealed recurrent UV-related *KNSTRN* gene mutations in 19% cSCC. *KNSTRN* encodes a kinetochore protein and mutations, particularly those encoding pSer24phe, disrupt chromatid cohesion in normal cells, correlate with aneuploidy in clinical samples and enhance tumourigenesis in a mouse model of human *RAS*-driven SCC. Mutations were also found in 13% AK, i.e. as with *TP53* and *NOTCH*, they appear to be an early event in squamous carcinogenesis.

Driver mutations are apparently tolerated in normal skin

Ultra-deep genome sequencing of normal eyelid skin has led to a paradigm shift in our understanding of squamous carcinogenesis (57). These researchers performed ultra-deep sequencing of 74 cancer genes in 234 tiny biopsies from normal eyelid skin from 4 individuals and confirmed a remarkably high level of somatic mutations in key genes, including *TP53*, *NOTCH 1-3*, *FGFR3*, *FAT1* and *RBM10*, and demonstrating tolerance of cancer-causing mutations in normal skin. Notch1 mutation was especially frequent found in up to 25% of normal keratinocytes and often in conjunction with LOH resulting in biallelic Notch1 inactivation (57). This raises many intriguing questions about the mechanisms/gatekeepers limiting progression to AK and to invasive cSCC.

cSCC metastatic progression

Although many studies have focused on the changes in early squamous cell carcinogenesis particularly occurring in AK and established invasive cSCC, there have been few studies of metastatic cSCC, yet this is an area of considerable unmet need for treatment. Early changes predominantly lead to loss of tumour suppressor function with the top 3 implicated genes being *TP53*, *NOTCH* family and *CDKN2A*. Recent studies of aggressive cSCC (58) and a study of lymph node metastases (52) identified a wide range of oncogenic drivers, with the majority of activating mutations in metastatic lesions being in RAS-RAF-MEK-ERK and PI3K/AKT pathways. It appears that loss of tumour suppressor function and resulting genomic instability lead to diverse oncogenic pathways being activated in metastatic progression. Although no clinically targetable dominant oncogenes were identified, it was suggested that, given the diversity of oncogenic targets, treatments currently available for other cancers might also be considered for advanced cSCC.

NEW TREATMENTS FOR SQUAMOUS CELL CARCINOMA: CURRENT LANDSCAPE AND FUTURE PERSPECTIVES

The impact of advances in genomic understanding of cancer has led to considerable clinical insights in predicting genetic susceptibility, cancer biomarker development and delivery of effective targeted therapies. However, targeting the important early loss of function mutations in *TP53* and *NOTCH* is challenging, although mouse studies have demonstrated a beneficial effect of p53 restoration in some tumours (59). Identification of aberrantly upregulated downstream or upstream proteins may ultimately prove more druggable targets. Undoubtedly, the area of most urgent clinical need is treatment for advanced cSCC not amenable to surgery or radiotherapy, which is potentially fatal and has few proven treatment options (60). Clinical activity has been shown in trials of various agents, including cytotoxic chemotherapies (cisplatin/carboplatin, 5-fluorouracil and its precursor drug capecitabine, bleomycin, doxorubicin, taxanes), 13-cis-retinoic acid, and interferon-α2a, but the significant limitations of these trials – small sizes, heterogeneous patient populations, and lack of randomization – has inevitably also limited their influence in defining treatment paradigms. The hope is that targeted therapies may provide a more effective alternative to treatments currently available. The diversity of activating oncogenic mutations in advanced disease suggests examining available therapies to multiple targets for potential efficacy in cSCC would be a logical therapeutic strategy.

Epidermal growth factor receptor (EGFR) inhibitors

EGFR inhibitors, approved for other cancers, are an attractive approach in view of the evidence for overexpression of EGFR in cSCC. Targeting EGFR inhibits the PI3K-AKT and RAS-RAF-ERK signal transduction pathways that regulate key cellular functions: although RAS mutation could theoretically overcome this effect by constitutively activating the downstream pathways independent of EGFR (as could *BRAF* mutations), most cSCC are RAS wild type (8). There are two classes of EGFR inhibitor: monoclonal antibodies that block the extracellular domain of the receptor and competitively inhibit EGFR (e.g. cetuzimab, panitumumab, nimotuzumab, zalutumumab); and small molecule tyrosine kinase inhibitors (TKIs), which block the activity of the tyrosine kinase ATP binding site and thereby inactivate downstream cellular pathways (e.g. gefitinib, erlotinib, afatinib, lapatinib, neratinib, dacomitinib). Both classes

are approved for use in cancers including lung, colorectal, HNSCC, pancreatic and breast cancer and have also been deployed in clinical trials of treatment for advanced cSCC with some evidence of clinical benefit.

EGFR monoclonal antibody inhibitors. Cetuximab is the most studied targeted therapy in cSCC (42). It is a chimeric mouse–human anti-EGFR IgG1 monoclonal antibody, which binds the extracellular EGFR domain with the same affinity as its natural ligands EGF and TGF-α and prevents dimerization of the receptor and downstream signaling. In addition, it inhibits nuclear EGFR transport, induces antibody-dependent cellular cytotoxicity and suppresses DNA-dependent protein kinase (61). Experience has been gained in locally advanced and/or recurrent/metastatic HNSCC and colorectal cancer. In HNSCC overexpression of EFGR predicts response to treatment and the presence of an extracellular domain deletion, EGFRvIII, predicts failure to respond, as in glioblastoma multiforme (62). In cSCC, cetuximab has been used in the neoadjuvant, adjuvant, monotherapy and combined therapy settings. There are few RCTs of its use in cSCC, but case reports report benefit in most. In a prospective phase II trial, Maubec et al. (63) were the first to demonstrate the efficacy of single agent cetuximab as first-line treatment for unresectable cSCC in 36 patients, with disease controlled at 6 weeks in 69% with a 28% response rate. Cetuximab has also been used in aggressive cSCCs arising in RDEB (64) and XP (65). Cetuximab is a known radiosensitiser and radiation enhancement of response is well established in HNSCC (66). It is also becoming apparent that immune mechanisms contribute to the clinical activity of cetuximab, with evidence of potentiation of both innate and adaptive immune responses against endogenous tumour antigens in HNSCC (67). There are case reports of adjuvant use of panitumab in cSCC and clinical trials are ongoing.

EGFR tyrosine kinase inhibitors. Currently approved EGFR tyrosine kinase inhibitors include gefitinib (non-small cell lung cancer), erlotinib (non-small cell lung and pancreatic cancer) and lapatinib (breast cancer). Their use in cSCC has been described in case reports and is the focus of several ongoing clinical trials. In contrast to lung cancer, few predictive biomarkers of response have been identified in either HNSCC or cSCC. In a phase II trial of neoadjuvent gefitinib prior to surgery or radiotherapy in 23 patients with unresectable cSCC, response rates of 45.5% (complete and partial response rates of 18% and 27%, respectively) were reported (68), and a phase I toxicity study of erlotinib combined with radiotherapy in 15 patients with advanced and recurrent cSCC demonstrated an acceptable toxicity profile and two-year recurrence rate and overall survival of 27% and 65% (69). Phase II trials of lapatinib in primary cSCC, neoadjuvant erlotinib,

and dacomitinib in unresectable or metastatic cSCC are in progress (www.clinicaltrials.gov).

EGFR inhibition combined with other targeted and non-targeted therapies. Primary resistance to EGFR inhibition may result from specific mutations, the most common of which is EGFRvIII mutation in HNSCC. It may also develop by increased receptor signaling resulting from ligand-independent receptor homo- and heterodimerization, bypassing EGFR inhibition (e.g. increased expression of EGFR, HER2, HER3, IGFR-1, and cMet). Simultaneous targeting of both EGFR and its binding partners such as HER-2 by trastuzumab, HER-3 by MM-121, IGFR-1 by cixutumumab and cMet by onartuzumab or ficlatuzumab may overcome this resistance (70). Preclinical and *in vivo* models also indicate that targeting the PI3K/mTOR pathway may rescue EGFR inhibitor resistance (71). Other therapeutic approaches with EGFR inhibitors currently under evaluation in HNSCC, which might also have activity in advanced cSCC, include combinations of EGFR antibody with TKI inhibitors; EGFR with VEGFR inhibitors (such as pazopanib); EGFR inhibitors with immunomodulators (such as Toll-like receptors). In addition, there are multiple trials in progress evaluating combinations of EGFR inhibitors with chemotherapy (such as cisplatin and paclitaxel) and radiotherapy (www.clinicaltrials.gov).

RAS/MAPK, PI3K, mTOR and NF-kB pathway inhibitors

There are currently no approved direct inhibitors of oncogenic Ras signaling, which has long been regarded as an 'undruggable' target (72). However, inhibiting signaling pathways downstream of Ras has been an area of active clinical research and targeting of the MAPK pathway with small molecule inhibitors in metastatic melanoma has resulted in a paradigm shift in the management of this disease. More than 20 inhibitors are currently under clinical evaluation and research has particularly focused on the RAF–MEK–ERK and the PI3K-AKT-mTOR signalling cascades, which contribute to cell proliferation, differentiation, and survival. None of these inhibitors are yet in clinical trials specifically for cSCC, but this is an area of intense research activity in HNSCC. Synergistic effects of combined PI3K/MEK inhibitors or PI3K/EGFR inhibitors have been observed pre-clinically in HNSCC and suggest that dual inhibition may be more effective, probably because compensatory signaling may result in only partial tumour growth inhibition when targeting either pathway alone. Preclinical and *in vivo* data also suggest that antibody to shed E-cadherin extracellular domain fragment, which suppresses cSCC growth via mechanisms which include upregulated MAPK and PI3K/AKT/mTOR signaling, may also represent a new therapeutic target for cSCC (73).

mTOR inhibition. Downstream of the lipid kinase PI3K, the protein kinase mTOR shares an evolutionarily related kinase domain, and is a key regulatory intracellular kinase integrating proliferation, survival and angiogenic pathways (74). mTOR functions as an intracellular physiologic sensor of nutrients: mTOR complex-1 regulates mRNA translation initiation, thus controlling the rate of protein synthesis and mTOR blockade with inhibitors such as everolimus, sirolimus (rapamycin) and temsirolimus may inhibit tumour growth. Upregulation of the PI3K-AKT-mTOR pathway is emerging as an important deregulated pathway in HNSCC and there are currently a number of clinical trials in progress evaluating the activity of temsirolimus, sirolimus and everolimus in advanced HNSCC, either alone or in combination with radiotherapy or chemotherapy (75). As indicated above, the mTOR pathway has also been implicated in resistance to EGFR inhibitors and mTOR pathway inhibitors may produce a cooperative effect with EGFR inhibitors in preclinical studies, a therapeutic strategy now being tested in the clinical setting in HNSCC (www.clinicaltrials.gov).

A broader application for mTOR inhibitors in cSCC may potentially lie in chemoprevention as they have both immunosuppressive and antitumour properties (76). Use of mTOR inhibitors as immunosuppressants is associated with a reduced incidence of malignancy and reduced incidence of cSCC in OTRs converted to mTOR inhibitors from calcineurin inhibitors: 4 RCTs and retrospective case series have now confirmed such a switch reduces incidence of post-transplant SCC and suggests that conversion to mTOR inhibitors should be undertaken early, possibly after the first SCC (76, 77). A recent systematic review of 21 RCTs confirmed a 56% reduction in NMSC and 40% reduction in malignancies overall in patients converted to mTOR inhibitors, but also identified an increased risk of death (HR 1.4) which may bring into question their possible use in cSCC prevention (78).

Src family kinase (SFK) inhibition and other TKIs

Dasatinib is a multi-kinase inhibitor with targets that include SFKs, BCR/Abl, PDGFR and c-KIT; it is approved for chronic myelogenous leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia (79) and is in trial for unresectable or metastatic cSCC. *In vitro*, Fyn inhibition by dasatinib reduces cell migration and promotes cell-cell adhesion, two important features of the EMT phenotype and in an SKH1 mouse model, topical dasatinib inhibited Fyn activity and reduced total tumour burden following UV exposure (80). Nilotinib is also a BCR-ABL TKI and is in clinical trials in combination with EGFR inhibitors for HNSCC.

Other potential approaches to treating cSCC

Although genetic aberrations in keratinocytes are critically important, many aspects of cSCC development and maintenance depend upon immune surveillance by resident and circulating immune cells and interactions between cancer cells and the stroma surrounding malignant keratinocytes consisting of interstitial extracellular matrix and its cellular components – the tumour microenvironment (TME). Cells of diverse lineages are found in the cSCC TME and include infiltrating immune cells, cancer associated fibroblasts (CAF), myofibroblasts and vasculature. Substantial data demonstrate extensive 'cross-talk' between these elements involving multiple signaling pathways including TGFβ, Notch, Wnt/beta-catenin, Shh/gli3, PDGFC, PI3/AKT-mTOR and p63-FGFR2 (81). Whilst the TME contributes to inhibiting progression of cancer, shifts in the balance of these interactions appear to provide a 'permissive' environment for tumour cells to proliferate, escape host defences and metastasize and possible opportunities for therapeutic intervention (82). Thus, there are potential targets for therapy for cSCC in modulating the behaviour of the immune cells and other components of the tumour microenvironment.

Immune surveillance and immunologically based therapies

The prevalence of cSCC in patients who are iatrogenically immunosuppressed emphasizes the importance of cell-mediated immunity in cSCC development and it is likely 'immunoediting' is involved at all stages of cSCC progression (83). Effective immune eradication of cSCC requires intact CD8^{+} CD4^{+} Th1 function: priming of CD8 cytotoxic T lymphocytes by antigen-presenting cells (APCs) in lymph nodes followed by clonal expansion of activated T cells, which migrate to skin and eliminate target antigens. Priming of a T-cell immune response requires binding of the T-cell receptor (TCR) to the tumour antigen-MHC 1 complex on skin APCs (dendritic cells) and co-stimulatory signaling by the TCR co-receptor, CD28 and B-7 ligands (CD80 and CD86) on APCs. Activation of T cells is modulated by a balance of positive, co-stimulatory signalling by TNF receptor family members (including CD40, OX40, and CD137) versus negative, co-inhibitory signalling by cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1). Within lymph nodes, displacement of CD28 by CTLA-4 dampens the priming phase whereas binding of PD-1 receptor on effector T cells by its natural ligand PD-L1 on tumour tissues leads to T-cell apoptosis as a second immune checkpoint.

Recent work has shown that skin-resident and migratory immune cells may also be subverted to support rather than inhibit tumour survival and progression (84). This complexity has been examined in more detail in HNSCC (85) and there are parallels in cSCC, with distinct roles for Langerhans cells, plasmacytoid dendritic cells, tumour-associated macrophages, myeloid-derived suppressor cells, regulatory T lymphocytes (T-regs) and

mast cells (86). These immune cells produce VEGF-C, growth factors, matrix proteases, other chemokines and chemokine receptors that, together with immuno-regulatory cytokines such as TGFβ, IL-10, VEGF-A and TNFα, may promote cancer growth and progression. cSCC can however develop other mechanisms to inhibit and escape immune surveillance including down-regulation of antigen processing and presentation, under-expression of co-stimulatory receptors such as CD40, overexpression of co-inhibitory receptors such as CTLA-4 and PD-1, aberrant expression of Fas and Fas ligand pro-apoptotic proteins (87, 88). In OTRs, the immune cell components of the TME may be significantly different to sporadic cSCC: the density of inflammatory infiltrate is reduced and the profile of reduced CD4$^+$ and cytotoxic CD8$^+$ T cells and possibly increased T-regs (89) is predicted to lead to a 'permissive' TME with decreased immune surveillance. Evidence of genetic susceptibility to key immune regulatory mechanisms may also affect risk for cSCC and a population-based, case-control study has identified interacting effects of common variants in two genes involved in aspects of inflammation and immunity, *RNASEL* and *MIR146A* (90).

Targeting immune surveillance mechanisms in cSCC has precedent in previous clinical trials of the immunostimulatory cytokine, interferon alpha, in advanced and high-risk cSCC, usually in combination with retinoids, which have shown some evidence of activity systemically and intralesionally (91). Immunomodulation has also been employed in use of the toll-like receptor-7 agonist, imiquimod, in topical treatment of AK. However, in recent years, the pace of advance in immunotherapies has increased significantly, particularly with the introduction of immune checkpoint blockade inhibitors and with developments in tumour antigen vaccination-based approaches.

Immunotherapeutic monoclonal antibodies. Immune checkpoint receptor monoclonal antibody inhibitors have shown significant activity in melanoma and both anti-CTLA-4 (ipilimumab) and anti-PD-1/PD-L1 (nivolumab, pembrolizumab) therapies are now approved for metastatic disease. Although not yet explored either as an independent therapeutic strategy or in combination with standard cytotoxic and other targeted modalities in advanced cSCC, such trials are currently ongoing in HNSCC (92). Monoclonal antibodies to immunosuppressive cytokines such as VEGF, TGFβ and HGF are also being explored, as are agonist monoclonal antibodies to co-stimulatory TNF receptor superfamily receptors such as CD40, OX40 and CD137. Whether such strategies will also be applicable to cSCC remains to be seen.

Vaccine-based therapies. Anti-tumour vaccines composed of tumour-specific antigens remain investigational. Potential strategies include peptide-based and whole tumour vaccines in HNSCC (93). Dendritic cells (DC)

including epidermal Langerhans cells-based approaches are a promising option in the skin, although the immunosuppressive microenvironment in cSCC must be overcome (88). Several methods have been used to deliver tumour-specific antigens directly to DCs, including coupling candidate antigens to antibodies targeting DC cell surface proteins (*in vivo* vaccination), harvesting peripheral DCs and activating them by exposure to candidate tumour antigen prior to re-infusion (*in vitro* vaccination) and direct application of candidate antigen to a disruption in the skin barrier which preferentially targets Langerhans cells (epicutaneous immunization). Possible antigenic candidates might be highly personalized, but equally may be more generally applicable, such as mutated TP53 and NOTCH as candidates given the importance of these genes in the development of many sporadic cSCC; HPV-based antigens may in the future be relevant if a more definitive role is confirmed in cSCC (93).

Other tumour microenvironment therapeutic targets

Cancer associated fibroblasts (CAFs). CAFs are the main cell type present in cSCC stroma and provide structural and biochemical support during development of cSCC, recruit macrophages, provide oncogenic signals such as fibroblast growth factors, and contribute to invasion via stromal–epithelial interactions (94). For example, mRNA expression profiling in RDEB has shown that downregulation of COL7A1 switches gene expression in dermal fibroblasts toward a CAF phenotype and the composition of fibroblast-derived ECM promotes substrate adhesion and invasion of tumour keratinocytes and tumour progression *in vivo* and is effectively 'permissive' to metastasis of the highly aggressive cSCCs that characterize RDEB (95). In XP-C dermal fibroblasts were thought to promote skin cancer via increased metalloproteinase activity (96). UVA was recently shown to inactivate Notch signaling in dermal fibroblasts by DNA methylation resulting in activation of CAFs with the production of growth factors and matrix proteins, which increase epithelial proliferation and are thought to underlie the generation of field cancerization in this model and potentially in sporadic cSCC (97). Recent studies indicate a TGFβ1-LIF-Jak/STAT3 tumour to CAF signaling cascade can drive tumour invasion in many epithelial cancers and may potentially be operative in cSCC (98).

Extracellular matrix, basement membrane zone, adhesion molecules and proteases. Tumour cell interactions with extracellular matrix (ECM) and basement membrane zone (BMZ) components play important roles in cSCC progression and metastasis. Adhesion molecules including integrins and cadherins mediate changes in cell-matrix and cell–cell interactions, respectively and are important in cSCC tumour cell migration, invasion and metastasis. Integrins link matrix proteins to the cytoskeleton in focal adhesions and changes in expression, mutation and loca-

lization impact on SCC development (99). Loss of kindlin-1, which binds to and activates integrin b6 subunits in hair follicle stem cells, induces skin tumours though both TGFß and wnt-b-catenin mediated mechanisms (100). E-cadherin promotes cell–cell adhesion at adherens junctions and may suppress tumour invasion (101): it is downregulated in more aggressive acantholytic variants of cSCC (102) and by type VII collagen in RDEB SCC keratinocytes (103). Loss of function is associated with cSCC progression, metastasis and poor prognosis (104). EMT, in which cells undergo transition from a polarized epithelial to motile mesenchymal phenotype by losing cell–cell adhesion properties, is important in invasion and cadherins are among the important mediators of EMT.

Proteases in the cSCC TME have multiple roles in tumour promotion, including inflammation and degradation/remodeling of the extracellular matrix tissue. These include matrix metalloproteinases (MMPs), inhibitors of MMPs (TIMPs) and a disintegrin and metalloproteinase (ADAM) family members, e.g. ADAM 10 and 17 (105). For example, a study in which the leading edge of cSCC were microdissected and analysed by cDNA microarray has shown an increased expression of MMP7: this may prove an interesting target as MMP7 increases proliferation, migration, and invasion of cancer cells and its inhibition delays tumour cell migration (106).

Many of these components and processes within the cSCC TME are potential therapeutic targets and therapies that target the TME or act both on tumour cells and stromal cells have already been approved for treatment of several cancers and trials are ongoing in HNSCC. For example, the angiogenesis inhibitor, bevacizumab is being explored in various combinations with chemotherapy, radiotherapy and cetuximab (107). Other drugs in clinical trials include the anti-inflammatory agent celecoxib, which is in phase 2 trials for HNSCC (108) in combination with erlotinib and chemoradiotherapy. Phosphodiesterase inhibitors such as tadalafil may reduce the immunosuppressive properties of MDSCs and Tregs and are being investigated in HNSCC. Integrin inhibitors such as cilengitide have been examined in combination with chemotherapy and cetuximab in advanced HNSCC (109). Matrix metalloproteinase inhibitors have been extensively investigated but have proved largely unsuccessful (82). Other drugs, particularly multikinase inhibitors which inhibit tumor cell growth pathways (e.g, BRAF, Bcr-Abl and c-Kit), also inhibit signaling from the microenvironment (e.g. VEGFR-1/2/3, PDGFR). Although further research is required in order to better understand the intricacies of the cSCC tumour microenvironment, such a combinatorial approach in cSCC may be a plausible therapeutic strategy.

Epigenetic alterations and potential therapies in cSCC

Epigenetic changes including DNA methylation, histone acetylation and the activity of microRNAs, can all change gene expression without any changes in the genetic sequence. Unlike genes that are inactivated by nucleotide sequence variation, genes silenced by epigenetic mechanisms remain intact and retain the potential to be reactivated by environmental or medical intervention (110). The contribution of epigenetic alterations in cSCC has received increasing attention in recent years, although published data remain limited.

Gene promotor methylation. Studies of methylation of individual genes have shown that promoter methylation of CDKN2A (p14ARF and p16INK4A) occurs in 40% of cSCC (48) and aberrant methylation of FOXE1 promoter has also been reported (111). FOXM1 is upregulated in cSCC and HNSCC and is associated with genome wide methylome changes and in HNSCC is associated with a specific signature of differentially methylated genes (112). Differential methylation was identified in the promoter region of FRZB, the protein product of which is an antagonist of Wnt signaling, in a study comparing methylation profiles of metastatic cSCC and non-metastatic primary cSCCs (113). Preliminary data also support a role for histone modifications in UV-induced skin carcinogenesis (114).

In contrast to DNA mutations, epigenetic mechanisms of squamous carcinogenesis may be reversible and therefore potentially amenable to therapeutic intervention. Drugs affecting or altering epigenetic regulation already have an established role in cancer: demethylating agents including the DNA methyltransferase inhibitors decitabine and 5-azacytidine are currently approved in haematological cancers, histone deacetylases inhibitors, vorinostat and romidespin are approved for cutaneous T-cell lymphoma and many more agents have been assessed or are currently being tested in preclinical trials. These agents may potentially be beneficial for treatment of cSCC, but none are yet in clinical trial. For example, HDACs are important regulators of p53 and p63 in skin (115) and vorinostat has shown activity in a human xenograft model of cSCC, possibly through mechanisms including inhibition of AKT/mTOR signaling and reduction in EMT by E-cadherin upregulation (116). Trials of epigenetic therapies are ongoing in HNSCC using, for example, vorinostat in combination with EGFR inhibitors and capecitabine (117), romidepsin and 5-azacytidine.

MicroRNA alterations. MicroRNAs (mIRs) are short non-coding RNAs that negatively regulate protein expression and mutations/alterations in miRs have been found in various cancers regulating cancer-associated pathways. Reported changes in miRs in cSCC include increased miR 21 (118) and downregulation of miR203, an antagonist of p63 (119), which is associated with metastasis in HNSCC. miR 124 and 214 are downregulated in cSCC which may contribute to overexpression of ERK1/2 and cellular proliferation in cSCC (120). miR 365 is overexpressed and may act as an

onco-miR (121). mIr 125-b is down-regulated in early cSCC and suppresses growth and motility of tumour cells through a network of pro-tumourigenic genes including matrix metallo-proteinases MMP13, MMP7 and MAP2K7 (122), whereas miR-135b was shown to be overexpressed in cSCC leading to increased cancer cell motility and invasiveness (123). However, there is relatively low consensus between studies; as with transcriptional profiling studies, this may be in part methodological. Long non-coding RNAs (lncRNAs) are also now increasingly recognised to play an important role in normal skin homeostasis (124) and TINCR, a lncRNA highly induced during keratinocyte differentiation, is repressed in cSCC, suggesting a role in repressing neoplastic progression in otherwise predisposed keratinocytes (125).

MiRNAs regulate multiple target genes simultaneously and may therefore represent promising therapeutic targets. Development of microRNA therapeutics has gathered pace in recent years and multiple agents are currently in preclinical trials for a range of cancers including hepatocellular cancer and glioblastoma and may have potential activity in HNSCC and cSCC, although this has yet to be explored (126).

Tumour heterogeneity and cancer stem cells: implications for targeted therapies

Increasingly sophisticated techniques for analysing cancer genomes are revealing that most cancers including cSCC are characterized by considerable genetic heterogeneity. Detailed analysis of multiple samples taken from individual primary and metastatic lesions of several cancer types has demonstrated a highly complex, non-linear, branching clonal evolutionary model as the basis for cancer progression and intratumoral heterogeneity (127, 128). This heterogeneity may result from diverse selective pressures including those related to host genome instability, epigenetic, tumour microenvironment and immunologic factors as well as therapeutic interventions and raises important issues, particularly in terms of discovery and also in terms of selection, response and resistance to targeted interventions. Early genetic ('truncal') events present in the majority of tumour cells are likely to be detected irrespective of the site sampled, but subsequent ('branch') events restricted to smaller clones may not be detected unless the tumour is extensively sampled. In terms of therapy, tumour sampling may influence whether all actionable genetic alterations are detected, and therefore whether the most appropriate therapy is offered to patients. Conversely, genetic alterations that confer resistance may not be detected if present only in specific clones within a tumour mass, potentially leading to inappropriate deployment of targeted therapies.

A further cause of variable responses to therapy resides in the concept of cancer stem cells and whether they can be found in cSCC. Studies to date have suggested that mutations in both follicular and interfollicular stem cells can give rise to cSCC, but also that tumours can express keratinocyte stem cell markers (129). Recent evidence implicates TGFβ signaling as a potent regulator of cancer stem cells and a potential driver of tumour heterogeneity experimentally in mice (54) warranting further dissection of TGFβ signaling in human cSCC in this context. These factors contributing to tumour heterogeneity could lead to different resistance mechanisms to single targeted therapies and would support a multifaceted approach.

Targeted therapies: repurposing of established drugs in cSCC

Re-purposing of established drugs may be an additional approach to targeted therapy for cSCC. Metformin is a biguanide used in treatment of type II diabetes and, in epidemiological studies, has been associated with a reduction in a range of cancers, including HNSCC (130). Preclinical evidence suggests that it may prevent squamous carcinogenesis by activating AMP kinase, which leads to inhibition of MAPK, PI3K, NF-κb and mTOR signaling pathways (131). The statin, lovastatin, is a mevalonate synthesis inhibitor and also targets AMP kinase: it may have activity in HNSCC as a single agent or synergise with EGFR inhibitors (132): other statins are now in clinical trials in combination with EGFR inhibitors for advanced cancers including HNSCC.

Targeted therapies for cSCC: potential challenges and future directions

Although there has been significant progress in recent years in understanding the molecular and genetic basis of cutaneous squamous carcinogenesis, much remains uncertain in comparison with less common epithelial tumours. The emerging landscape of genomic and epigenomic alterations underscores the high mutational burden, dysregulation of multiple signaling pathways and tumour heterogeneity associated with cSCC, and has proved challenging in terms of identifying novel drivers and predictive biomarkers for targeted therapies. Most evidence to date relating to targeted therapies for cSCC has focused on EGFR inhibition and mTOR pathway signal transduction blockade. Targeting of other potential candidates including p53, NOTCH and MAPK pathway may prove to be effective in cSCC, but most are currently further away from routine clinical use, as are epigenetic approaches. Ultimately, however, it is likely that multi-pathway/modality approaches will provide the most effective 'personalized' therapeutic strategies, particularly for advanced cSCC, as is clearly

now emerging for melanoma. Given the critical importance of the tumour microenvironment and immune system, strategies incorporating therapies targeting these components of the cSCC landscape must also be considered. Such treatments are already being applied, or are becoming available, in HNSCC and other cancers. However, although there are many ongoing preclinical studies in cSCC, clinical studies are limited: for example, in a search of metastatic cutaneous SCC on the www. clinicaltrials.gov website, only 3 trials are currently registered, in contrast to excess of one thousand for metastatic melanoma. Nonetheless, the prospect of 'precision medicine' characterized by molecular profiling of cSCC to identify the relevant molecular alterations, followed by biomarker-driven selection of optimal, individualized therapies is now at least on the horizon.

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REFERENCES

1. Guy GP, Jr., Machlin SR, Ekwueme DU, Yabrof KR. Prevalence and costs of skin cancer treatment in the US; 2002–2006 and 2007–2011. Am J Prev Med 2015; 48 183–187.
2. Weinberg AS, Ogle CA, Shim EK. Metastatic cutaneous squamous cell carcinoma: an update. Dermatol Surg 2007; 33: 885–899.
3. Mavropoulos JC, Aldabagh B, Arron ST. Prospects for personalized targeted therapies for cutaneous squamous cell carcinoma. Semin Cutan Med Surg 2014; 33: 72–75.
4. Hutchinson L. Skin cancer: Setting the stage for cutaneous SCC. Nat Rev Clin Oncol 2014; 11: 63.
5. Jambusaria-Pahlajani A, Kanetsky P, Karia PS, Hwang W, Gelfand JM, Whalen FM, et al. Evaluation of AJCC tumor (T) staging for cutaneous squamous cell carcinoma and a proposed alternative tumor staging system. JAMA Dermatol 2013; 16: 1–9.
6. Carucci JA. Press for an underestimated nemesis. JAMA Dermatol 2013; 149: 1147–1148.
7. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J Am Acad Dermatol. 2013; 68: 957–966.
8. South AP, Purdie KJ, Watt SA, Haldenby S, den Breems NY, Dimon M, et al. NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. J Invest Dermatol 2014; 134: 2630–2638.
9. Fine JD, Johnson LB, Weiner M, Li KP, Suchindran C Epidermolysis bullosa and the risk of life-threatening cancers: the National EB Registry experience, 1986–2006. J Am Acad Dermatol 2009; 60: 203–211.
10. Goudie DR, D'Alessandro M, Merriman B, Lee H, Szeverényi I, Avery S, et al. Multiple self-healing squamous epithelioma is caused by a disease-specific spectrum of mutations in TGFBR1. Nat Genet 2011; 43: 365–369.
11. Binstock M, Hafeez F, Metchnikoff C, Arron ST. Single-nucleotide polymorphisms in pigment genes and non melanoma skin cancer predisposition: a systematic review

Br J Dermatol 2014; 171: 713–721.
12. Ruczinski I, Jorgensen TJ, Shugart YY, Schaad YB, Kessing B, Hoffman-Bolton J, et al. A population-based study of DNA repair gene variants in relation to non-melanoma skin cancer as a marker of a cancer-prone phenotype. Carcinogenesis 2012; 33: 1692–1698.
13. McGregor JM, Harwood CA, Brooks L, Fisher SA, Kelly DA, O'Nions J, et al. Relationship between p53 codon 72 polymorphism and susceptibility to sunburn and skin cancer. J Invest Dermatol 2002; 119: 84–90.
14. Laing ME, Kay E, Conlon P, Murphy GM. Genetic factors associated with skin cancer in renal transplant patients. Photodermatol Photoimmunol Photomed 2007; 23: 62–67.
15. Harwood CA, Mesher D, McGregor JM, Mitchell L, Leedham-Green M, Raftery M, et al. A surveillance model for skin cancer in organ transplant recipients a 22 year prospective study in an ethnically diverse population. Am J Transplant 2013; 13: 119–129.
16. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013; 339: 1546–1558.
17. Stahl PL, Stranneheim H, Asplund A, Berglund L, Ponten F, Lundeberg J. Sun-induced non-synonymous p53 mutations are extensively accumulated and tolerated in normal appearing human skin. J Invest Dermatol 2011; 131: 504–508.
18. Purdie KJ, Harwood CA, Gulati A, Chaplin T, Lambert SR, Cerio R, et al. Single nucleotide polymorphism array analysis defines a specific genetic fingerprint for well-differentiated cutaneous SCCs. J Invest Dermatol 2009; 129: 1562–1568.
19. Boukamp P. Non-melanoma skin cancer: what drives tumor development and progression? Carcinogenesis 2005; 26: 1657–1667.
20. Purdie KJ, Lambert S, Teh MT, Chaplin T, Molloy G, Raghavan M, et al. Allelic imbalances and micro-deletions affecting the PTPRD gene in cutaneous squamous cell carcinomas detected using single nucleotide polymorphism microarray analysis. Genes Chromosomes Cancer 2007; 46: 661–669.
21. Lambert SR, Harwood CA, Purdie KJ, Gulati A, Matin RN, Romanowska M, et al. Metastatic cutaneous squamous cell carcinoma shows frequent deletion in the protein tyrosine phosphatase receptor D gene. Int J Cancer 2012: 131; 216–226.
22. Leufke C, Leykauf J, Krunic D, Jauch A, Hotlgreve-Grez H, Böhm-Steuer B, et al. The telomere profile distinguishes two classes of genetically distinct cutaneous squamous cell carcinomas. Oncogene 2014; 33: 3506–3518.
23. Tomasetti C, Vogelstein B. Only three driver mutations are required for the development of lung and colorectal cancers. Proc Natl Acad Sci USA 2015; 112: 118–123.
24. Bignell GR, Greenman CD, Davies H, Butler AP, Edkins S, Andrews JM, et al. Signatures of mutation and selection in the cancer genome. Nature 2010; 463: 893–898.
25. Giglia-Mari G, Sarasin A.TP53 mutations in human skin cancers. Hum Mutat 2003; 21: 217–228.
26. Jonason AS, Kunala S, Price GJ, Restifo RJ, Spinelli HM, Persing JA, et al. Frequent clones of muatted p53 in normal skin. Proc Natl Acad Sci USA 1996; 93: 14025–14029.
27. Zhang W, Hanks AN, Boucher K, Florell SR, Allen SM, Alexander A, et al. UVB-induced apoptosis drives clonal expansion during skin tumor development. Carcinogenesis 2005; 26: 249–257.
28. Durinck S, Ho S, Wang NJ, Liao W, Jakkula LR, Collisson EA, et al. Temporal dissection of tumorigenesis in primary cancers. Cancer Discov 2011; 1: 137–143.

29. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci U S A 2011; 108: 17761–17766.

30. South AP, Cho RJ, Aster JC. The double-edged sword of Notch signaling in cancer. Semin Cell Dev Biol 2012; 23: 458–464.

31. Stransky N, Egloff NM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science 2011; 333: 1157–1160.

32. Rangarajan A, Talora C, Okuyama R, Nicolas M, Mammucari C, Oh H, et al. Notch signaling is a direct determinant of keratinocyte growth arrest amd entry into differentiation. EMBO J 2001; 20: 3427–3436.

33. Restivo G, Nguyen BC, Dziunycz P, Ristorcelli E, Ryan RJ, Özuysal ÖY, et al. IRF6 is a mediator of Notch pro-differentiation and tumour suppressive function in keratinocytes. EMBO J 2011; 30: 4571–4585.

34. Nguyen BC, Lefort K, Mandinova A, Antonini D, Devgan V, Della Gatta G, et al. Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. Genes Dev 2006; 20: 1028–1042.

35. Balmain A, Ramsden M, Bowden GT, Smith J. Activation of the mouse cellular Harvey-ras gene in chemically induced benign skin papillomas. Nature 1984; 307: 658–680.

36. Lazarov M, Kubo Y, Cai T, Dajee M, Tarutani M, Lin Q, et al. CDK4 co-expression with Ras generates malignant human epidermal tumorigenesis. Nat Med 2002; 8: 1105–1114.

37. Dajee M, Lazarov M, Zhang JY, Cai T, Green CL Russell AJ, et al. NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. Nature 2003; 421: 639–643.

38. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012; 366; 207–215.

39. Iglesias-Bartholome R, Martin D, Gutkind JS. Exploiting the head and neck cancer oncogenome: widespread PI3K-mTOR pathway alterations and novel molecular targets. Cancer Discov 2013; 3: 722–725.

40. Lambert SR, Mladkova N, Gulati A, Hamoudi R, Purdie K, Cerio R, et al. Key differences identified between actinic keratosis and cutaneous squamous cell carcinoma by transcriptome profiling. Br J Cancer 2014; 110: 520–529.

41. Ming M, He YY. PTEN new insights into its regulation and function in skin cancer. J Invest Dermatol 2009; 129: 2109–2112.

42. Uribe P, Gonzalez S. Epidermal growth factor receptor (EGFR) and squamous cell carcinoma of the skin: molecular bases for EGFR-targeted therapy. Pathol Res Pract 2011; 207: 337–342.

43. Kolev V, Mandinova A, Guinea-Viniegra J, Hu B, Lefort K, Lambertini C, et al. EGFR signaling as a negative regulator of Notch1 gene transcription and function in proliferating keratinocytes and cancer. Nat Cell Biol 2008; 10: 902–911.

44. Toll A, Salgado R, Yebenes M, Martin-Ezquerra G, Gliaberte M, Baro F, et al. Epidermal growth factor receptor gene numerical aberrations are frequent events in actinic keratosis and invasive squamous cell carcinoma. Exp Dermatol 2010; 19: 151–153.

45. Zandi R, Larsen AB, Andersen P, Stockhausen MT, Poulsen HS. Mechanisms for oncogenic activation of the epidermal growth factor receptor. Cell Signal 2007; 19: 2013–2023.

46. Brunton VG, Frame MC. Src and focal adhesion kinase as therapeutic targets in cancer. Curr Opin Pharmacol 2008; 8: 427–432.

47. Zhao L, Li W, Marshall C, Griffin T, Hanson M, Hick R, et al. Srcasm inhibits Fyn-induced cutaneous carcinogenesis with modulation of Notch1 and p53. Cancer Res 2009; 69: 9439–9447.

48. Brown VL, Harwood CA, Crook T, Cronin JG, Kelsell DP, Proby CM. p16INK4a and p14ARF tumor suppressor genes are commonly inactivated in cutaneous squamous cell carcinoma. J Invest Dermatol 2004: 122: 1284–1292.

49. Dixit V, Mak TW. NF-kappaB signaling. Many roads lead to Madrid. Cell 2002; 111: 615–619.

50. Poligone B, Hayden MS, Chen L, Pentland AP, Jimi E, Ghosh S. A role for NF-kB activity in skin hyperplasia and the development of keratoacanthomata in mice. PLOS One 2013; 8: e71887.

51. Watt SA, Purdie KJ, Y den Breems N, Dimon M, Arron ST, McHugh A, et al. Novel CARD11 mutations in human cutaneous squamous cell carcinoma lead to aberrant NFκB regulation. Am J Pathol 2015 Jul 23 [Epub ahead of print].

52. Li YY, Hanna GJ, Laga AC, Haadad RI, Lorch JH, Hammerman PS. Genomic analysis of metastatic cutaneous squamous cell carcinoma. Clin Cancer Res 2015; 21: 1447–1456.

53. Lacouture ME, Morris JC, Lawrence DP, Tan AR, Olencki TE, Shapiro GI, et al. Cutaneous keratoacanthomas/squamous cell carcinomas associated with neutralization of transforming growth factor β by the monoclonal antibody fresolimumab (GC1008). Cancer Immunol Immunother 2015; 64: 437–446.

54. Inman GJ. Switching TGF beta from a tumor suppressor to a tumor promoter. Curr Opin Genet Dev 2011; 21: 93–99.

55. Oshimori N, Onshan D, Fuchs E. TGF beta promotes heterogeneity and drug resistance in cSCC. Cell 2015; 160: 963–976.

56. Lee CS, Bhaduri A, Mah A, Johnson WL, Ungewickell A, Aros CJ, et al. Recurrent point mutations in the kinetochore gene KNSTRN in cutaneous squamous cell carcinoma. Nat Genet 2014; 46: 1060–1062.

57. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, Wedge DC, Fullam A, Alexandrov LB, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 2015; 348: 880–886.

58. Pickering CR, Zhou JH, Lee J, Drummond JA, Peng A, Saade RE, et al. Mutational landscape of aggressive squamous cell carcinoma. Clin Cancer Res 2015; 20: 3842–3848.

59. Feldser DM, Kostova KK, Winslow MM, Taylor SE, Cashman C, Whittaker CA, et al. Stage-specific sensitivity to p53 restoration during lung cancer progression. Nature 2010; 468: 572–575.

60. Bejar C, Maubec E. Therapy of advanced squamous cell carcinoma of the skin. Curr Treat Options Oncol 2014; 15: 302–320.

61. Rabinowitz G, Haddad RI. Overcoming resistance to EGFR inhibitor in head and neck cancer a review of the literature. Oral Oncol 2012; 48: 1085–1089.

62. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors, N. Engl J Med 2005; 353: 2012–2024.

63. Maubec E, Petrow P., Scheer-Senyarich I, Duvillard P, Lacroix L, Gelly J, et al. Phase II study of cetuximab as first-line single-drug therapy in patients with unresectable squamous cell carcinoma of the skin. J Clin Oncol 2011;

29: 3419–3426.

64. Arnold AW, Bruckner-Tuderman L, Zuger C, Itin PH. Cetuximab therapy of metastasizing cutaneous squamous cell carcinoma in a patient with severe recessive dystrophic epidermolysis bullosa. Dermatology 2009; 219: 80–83.

65. Rubio-Casadevall J, Grana-Suarez B, Hernandez-Yague X, Vayreda Ribera J, Huc Grasa O, Brunet Vidal J. Xeroderma pigmentosum: Neck lymph node metastasis of a squamous cell carcinoma of the skin treated with cetuximab. Eur J Dermatol 2009 19: 163–165.

66. Bonner JA, Harari PM, Girait J, Azamia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, N Engl J Med 2006; 354: 567–578.

67. Srivastava RM, Lee SC, Andrade Filho PA, Lord CA, Jie HB, Davidson HC, et al. Cetuximab-activated natural killer and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients. Clin Cancer Res 2013; 19: 1858–1872.

68. Lewis CM, Glisson BS, Feng L, Wan F, Tang X, Wistuba II, et al. A phase II study of gefitinib for aggressive squamous cell carcinoma of the head and neck. Clin Cancer Res 2012; 18: 1435–1446.

69. Heath CH, Deep NL, Baell L, Carroll WR, Desmond R, Clemons L, et al. Phase 1 study of erlotinib plus radiation therapy in patients with advanced cutaneous squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2013; 85: 1275–1281.

70. Bossi P, Lo Vullo S, Guzzo M, Mariani L, Granata R, Orlandi E, et al. Emerging tyrosine kinase inhibitors for head and neck cancer. Expert Opin Emerg Drugs 2013; 18: 445–459.

71. D'Amato V, Rosa R, D'Amato C, Formisano L, Marciano R, Nappi L, et al. The dual PI3K/mTOR inhibitor PKI-587 enhances sensitivity to cetuximab in EGFr resistant human head and neck cancer models. Br J Cancer 2014; 110: 2887–2895.

72. Baker NM, Der CJ. Drug for an 'undruggable' protein. Nature 2013; 497: 577–578.

73. Brouxhon SM, Kykanides S, Teng X, Athar M, Ghazizadeh S, Simon M et al. Solubel E-cadherin: a critical oncogene modulating receptor tyrosine kinases, MAPK and PI3K/Akt/mTOR signaling. Oncogene 2014; 33: 225–235.

74. de Gruijl FR, Koehl GE, Voskamp P, Strik A, Rebel HG, Gaumann A, et al. Early and late effects of the immuno-suppressants rapamycin and mycophenolate mofetil on UV carcinogenesis. Int J Cancer 2010; 127: 796–804.

75. Mazumdar T, Byers LA, Ng PK, Mills GB, Peng S, Diao L, et al. A comprehensive evaluation of biomarkers predictive of response to PI3K inhibitors and of resistance mechanisms in head and neck squamous cell carcinoma. Mol Cancer Ther 2014 13: 2738–2750.

76. Hoogendijk-van den Akker JM, Harden PN, Hoitsma AJ, Proby CM, Wolterbeek R, Bouwes Bavinck JN, et al. Two-year randomised controlled prospective trial converting treatment of stable renal transplant recipients with cutaneous invasive squamous cell carcinomas to sirolimus. J Clin Oncol 2013; 31: 1317.

77. Euvrard S, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, et al. Sirolimus and secondary skin-cancer prevention in kidney transplantation. N Engl J Med 2012; 367: 329–339.

78. Knoll GA, Kokolo MB, Malick R, Beck A, Buenaventura CD, Ducharme R, et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. BMJ 2014; 349: g6679.

79. Gnoni A, Marech I, Silvestris N, Vacca A, Lorusso V. Dasatinib: An anti-tumour agent via Src inhibition. Curr Drug Targets 2011; 12: 563–578.

80. Fenton SE, Hutchens KA, Denning MF. Targeting Fyn in Ras-transformed cells induces F actin to promote adherens junction-mediated cell-cell adhesion. Mol Carcinog 2014 Jun 29. [Epub ahead of print].

81. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci 2012; 125: 5591–5596.

82. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role for the microenvironment in restraining cancer progression. Nat Med 2011 17: 320–329.

83. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002; 3: 991–998.

84. Rangwala S, Tsai KY. Roles of immune system in skin cancer. Br J Dermatol 2011; 165: 953–965.

85. Freiser ME, Serafini P, Weed DT. The immune system and head and neck squamous cell carcinoma: from carcinogenesis to new therapeutic opportunities. Immunol Res 2013; 57: 52–69.

86. Yanofsky VR, Mitsui H, Felsen D, Carucci JA. Understanding dendritic cells and their role in cutaneous carcinoma and cancer immunotherapy. Clin Dev Immunol 2013; 62: 412–413.

87. Satchell AC, Barnetson RS, Halliday GM. Increased Fas ligand expression by T cells and tumour cells in the progression of actinic keratosis to squamous cell carcinoma. Br J Dermatol 2004; 151: 42–49.

88. Lee YB, Kyung Kim E, Park HJ, Cho BK, Park YM, Kim JW, Y, et al. Expression of Fas and Fas ligand in primary cutaneous squamous cell carcinoma in association with grade of tumor differentiation. Int J Dermatol 2013; 52: 1092–1097.

89. Zhang S, Fujita H, Mitsui H, Yanofsky VR, Fuentes-Duculan J, Pettersen JS, et al. Increased Tc22 and Treg/CD8 ratio contribute to aggressive growth of transplant-associated squamous cell carcinoma. PLoS One 2013; 8: e62154.

90. Farzan SF, Karagas MR, Christensen BC, Li Z, Kuriger JK, Nelson HH; New Hampshire Skin Cancer Study. RNASEL and MIR146A SNP-SNP interaction as a susceptibility factor for non-melanoma skin cancer. PLoS One 2014; 9: e93602.

91. Brewster AM, Lee JJ, Clayman GL, Clifford JL, Reyes MJ, Zhou X, et al. Randomized trial of adjuvant 13-cis-retinoic acid and interferon alfa for patients with aggressive squamous cell carcinoma. J Clin Oncol 2007; 25: 1974–1978.

92. Bauman JE1, Ferris RL. Integrating novel therapeutic monoclonal antibodies into the management of head and neck cancer. Cancer 2014; 120: 624–632.

93. Gildener Leapman N, Lee J, Ferris RL. Tailored immunotherapy for HPV positive head and neck squamous cell cancer. Oral Oncol 2014 50: 780–784.

94. Lim YZ, South AP. Tumour-stroma cross talk in the development of squamous cell carcinoma. Int J Biochem Cell Biol 2014: 53: 450–458.

95. Ng YZ, Pourreyron C, Salas-Alanis JC, Dayal JH, Calpeda-Valdes R, Yan W, et al. Fibroblast derived dermal matrix drives development of aggressive squamous cell carcinoma in patients with recessive dystrophic epidermolysis bullosa. Cancer Res 2012; 72: 3522–3534.

96. Frechet M, Warrick E, Vioux C, Chevallier O, Spatz A, Benhamou S, et al. Overexpression of matrix metalloproteinases in dermal fibroblasts from DNA repair deficient/cancer-prone xeroderma pigmentosum group C patients. Oncogene 2008; 27: 5223–5232.

97. Hu B, Castillo E, Harewood L, Ostano P, Reymond A, Dummer R, Raffoul W, Hoetzenecker W, Hofbauer GF, Dotto GP. Multifocal epithelial tumors and field cancerization from loss of mesenchymal CSL signaling. Cell. 2012; 149: 1207–1220.

98. Albrengues J, Bourget I, Pons C, Butet V, Hofman P, Tartare-Deckert S, et al. LIF mediates proinvasive activation of stromal fibroblasts in cancer. Cell Rep 2014; 7: 1664–1678.

99. Maalouf SW, Theivakumar S, Owens DM. Epidermal a6b4 integrin stimulates the influx of immunosuppressive cells during skin tumor promotion. J Dermatol Sci 2012; 66: 108–118.

100. Rognoni E, Widmaier M, Jakobson M, Ruppert R, Ussar S, Katsougkri D, et al. Kindlin-1 controls Wnt and TGF-b availability to regulate cutaneous stem cell proliferation. Nat Med 2014; 20: 350–359.

101. Yap AS, Crampton MS, Hardin J. Making and breaking contacts: the cellular biology of cadherin regulation. Curr Opin Cell Biol 2007; 19: 508–514.

102. Griffin JR, Wriston CC, Peters MS, Lehman JS. Decreased expression of intercellular adhesion molecules in acantholytic squamous cell carcinoma with invasive well-differentiated squamous cell carcinoma of the skin. Am J Clin Pathol 2013; 139: 442–447.

103. Dayal JH, Cole CL, Pourreyron C, Watt SA, Lim YZ, Salas-Alanis JC, et al. Type VII collagen regulates expression of OATB1B3, promotes front to rear polarity and increases organization in 3D spheroid cultures of RDEB tumour keratinocytes. J Cell Sci 2014; 127: 740–751.

104. Behrens J. Cadherins and catenins: role in signal transduction and tumor progression. Cancer Metastasis Rev 1999; 18: 15–30.

105. Kessenbroek K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumour microenvironment. Cell 2010; 141: 52–67.

106. Mitsui H, Suarez-Farinas M, Gulati N, Shah KR, Canizzaro MV, Coats I, et al. Gene expression profiling of the leading edge of cutaneous squamous cell carcinoma: IL-24 driven MMP7. J Invest Dermatol 2014: 134: 1418–1427.

107. Yao M, Galanopoulos N, Lavertu P, Fu P, Gibson M, Argiris A, et al. Phase II study bevacizumab in combination with docetaxel and radiation in locally advanced squamous cell carcinoma of the head and neck. Head Neck 2014 Jun 21. [Epub ahead of print].

108. Kao J, Genden EM, Chen CT, Rivera M, Tong CC Misiukiewicz K, et al. Phase 1 trial of concurrent erlotinib, celecoxib and reirradiation for recurrent head and neck cancer. Cancer 2011; 117: 3172–3181.

109. Vermorken JB, Peyrade F, Krauss J, Mesia R, Remenar E, Gauler TC, et al. Cisplatin, 5-fluorouracil and cetuximab (PFE) with or without cilengitide in recurrent/metastatic squamous cell carcinoma of the head and neck: results of randomized phase I/II ADVANTAGE trial. Ann Oncol 2014: 25: 682–688.

110. Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. Trends Pharmacol Sci 2014; 35: 384–396.

111. Venza I, Visalli M, Tripodo B, De Grazia G, Loddo S, Teti D Venza M. FOXE1 is a target for aberrant methylation in cutaneous squamous cell carcinoma. Br J Dermatol 2010; 162: 1093–1097.

112. Teh MT, Hutchison IL, Costea DE, Neppelberg E, Liavaag PG, Purdie K, et al. Exploiting FOXM1-orchestrated molecular network for early squamous cell carcinoma diagnosis and prognosis. Int J Cancer 2013; 132: 2095–2106.

113. Darr OA, Colacino JA, Tang AL, McHugh JB, Bellile EL, Bradford CR, et al. Epigenetic alterations in metastatic cutaneous carcinoma. Head Neck 2015; 37(7): 994–1001.

114. Nandakumar V, Vaid M, Tollefsbol TO, Katiyar SK. Aberrant DNA hypermethylation patterns lead to transcriptional silencing of tumor suppressor genes in UVB-exposed skin and UVB-induced skin tumors of mice. Carcinogenesis 2011; 32: 597–604.

115. Ramsey MR, He L, Forster N,Ory B, Ellisen LW. Physical association of HDAC1 and HDC2 with p63 mediates transcriptional repression and tumor maintenance in squamous cell carcinoma. Cancer Res 2011; 71: 433–439.

116. Kurundkar D, Srivastava RK, Chaudhury SC, Ballestas ME, Kopelovich L, elmets CA, Athar M. Vorinostat, an HDAC inhibitor attenuates epidermoid squamous cell carcinoma growth by dampening mTOR signaling pathway in a human xenograft murine model. Toxicol Appl Pharmacol 2013; 266: 233–244.

117. Bruzzese F, Leone A, Rocco M, Carbone C, Piro G, Caraglia M. HDAC inhibitor vorinostat enhances the antitumor effect of gefitinib in squamous cell carcinoma of head and neck by modulating ErbB receptor expression and reverting EMT J Cell Physiol 2011; 9: 2378–2390.

118. Sand M, Skrygan M, Georgas D, Sand D, Hahn SA, Gambichler T, et al. Microarray analysis of microRNA expression in cutaneous squamous cell carcinoma. J Dermatol Sci 2012; 68: 119–126.

119. Dziuncyz P, Iotzova-Weiss G, Eloranta JJ, Lauchli S, Hafner J, French L, et al. Squamous cell carcinoma of the skin shows a distinct micro RNA profile modulated by UV radiation. J Invest Dermatol 2010; 130: 2686–2689.

120. Yamane K, Jinnin M, Etoh T, Kobayashi Y, Shimozono N, Fukushima S, et al. Down-regulation of miR-124/-214 in cutaneous squamous cell carcinoma mediates abnormal cell proliferation via the induction of ERK. J Mol Med (Berl) 2013; 91: 69–81.

121. Zhou M, Liu W, Ma S, Cao H, Peng X, Guo L, et al. A novel onco-miR-365 induces cutaneous squamous cell carcinoma. Carcinogenesis 2013 34: 1653–1659.

122. Xu N, Zhang L, Meisgen F, Harada M, Heilborn J, Homey B, et al. MicroRNA-125b down-regulates matrix metallopeptidase 13 and inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion. J Biol Chem 2012; 287: 29899–29908.

123. Olasz EB, Seline LN, Schock AM, Duncan NE, Lopez A, Lazar J, et al. MicroRNA-135b regulates leucine zipper tumor suppressor 1 in cutaneous squamous cell carcinoma. PLOS One2015 May 4; 10(5): e0125412.

124. Hombach S, Kretz M. The non coding skin: exploring the roles of long non-coding RNAs in epidermal homeostasis Bioessays 2013; 35: 1093–1100.

125. Kretz M. TINCR, staufen 1 and cellular differentiation RNA Biol 2013; 10: 1597–1601.

126. van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. EMBO Mol Med 2014; 6: 851–864.

127. de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science 2014; 346: 251–256.

128. Russnes HG, Lønning PE, Børresen-Dale AL, Lingjærde OC. The multitude of molecular analyses in cancer: the opening of Pandora's box. Genome Biol 2014; 15: 447.

129. Jensen KB, Jones J, Watt FM. A stem cell expression profile of human squamous cell carcinomas. Cancer Lett 2008; 272: 23–31.

130. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330: 1304–1305.

131. Ma L, Niknejad N, Gorn-Hondermann I, Dayekh K, Dimitroulakos J. Lovastatin induces multiple stress pathways including LKB1/AMPK activation that regulate its cytotoxic effects in squamous cell carcinoma cells. PLoS ONE 2012; 7: e46055.

132. Chaudhary SC, Kurundkar D, Elmets CA, Kopelovich L, Athar M. Metformin, an anti-diabetic agent reduces growth of cutaneous squamous cell carcinoma by targeting mTOR signaling pathway. Photochem Photobiol 2012; 88: 1149–1145.