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# Molecular characterization of vulvar squamous cell cancer: High time to gain ground

Vulvar squamous cell cancer (VSCC) is a rare disease, but its incidence shows a steady rise over the last three decades. Interestingly, this cancer consists of two etiological subtypes. One is associated with human papilloma virus (HPV) affecting younger women, whereas the other one is unrelated to HPV, develops from chronic dystrophic vulvar diseases and is more prevalent in older women. The HPV association in about 40% of these carcinomas is viewed as being responsible for the continuous increase in VSCC incidence worldwide. Despite the increasing numbers, the interesting dual pathogenesis of VSCC, the broad general commitment to precision or personal treatment strategies in human cancer and the rising availability of drugs enabling novel targeted therapies, vulvar cancer is one of those tumor entities, which remained unconsidered in the TCGA and therefore comprehensive molecular characterization of this cancer is lacking. However, an in depth understanding of the molecular biology is the prerequisite for opening new avenues for additional treatment options. All this, together with a relative paucity of clinical trials led Clancy et al. to dub the VSCC "the forgotten women's cancer" [1].

In order to significantly improve this unsatisfying situation, the genomic characterization of VSCC was addressed by Katharina Prieske et al. as a highlight of the current issue [2]. One strength of their investigations is, that whole exome sequencing (WES) was performed on the DNA of 34 VSCC and matched normal vulvar tissues were used as comparator for each patient. This is the largest and only the second study to make use of WES in VSCC, while the other reports either referred to Sanger sequencing or used next generation sequencing (NGS) analyses, but the latter were restricted to various panels comprising a limited number of cancer-specific hotspot genes. Although considerable differences in the mutational signatures have been revealed between the two now existing WES studies, both investigations provided clear-cut evidence to show that HPV-related and HPV-unrelated VSCC should in fact be regarded as two separate diseases [3]. Both studies pointed out the role of somatic *TP53* mutations as a pivotal oncogenic driver in HPV-unrelated cancers, as *TP53* alterations were highly prevalent and exclusively found in HPV-negative cancers. However, this mutual exclusivity between *TP53* mutations and HPV-positivity stands in contrast to an earlier report that described *TP53* mutations in HPV-related VSCC with an incidence of 17% [4]. On the other hand, in both WES studies a few HPV-unrelated cancers displayed a *TP53* wildtype status, thus allowing the conclusion that these cancers do not necessarily require a *TP53* alteration as principal oncogenic driver. The small sample size prevented both of the two WES approaches from either confirming or excluding the existence of this third "*double-negative*" VSCC type, that was previously postulated by the Dutch group of Nooij et al. [5] These authors found this third *TP-53*- and HPV-negative subgroup to be characterized by a predominance of *Notch1* and *HRAS* genomic alterations, each in 50% of the cases. Neither of this genomic alterations, however, has been detected at a noteworthy frequency in either of the WES-examined cohorts.

The E3 ubiquitin ligase *FBXW7* is another gene that was found to be somatically altered especially in HPV-unrelated VSCC. *FBXW7* mutations are of special interest as they may affect multiple important cellular pathways that strengthen the malignant potential of cancer cells, because the FBXW7 protein is crucially involved in the ubiquitination and cellular elimination of a large number of oncoproteins. Its loss of function by mutations leads to an indirect over-expression of its numerous clients, including cyclin-E, c-Myc and mTOR, conferring a high proliferative turn-over and an aggressive behavior on affected malignant cells. Furthermore, FBXW7 is a chief negative regulator of Notch1 and was therefore proposed by Prieske et al. as a possible substitute for *Notch1* mutations in VSCC [2].

One common key finding from all the so far existing studies on VSCC molecular characterization, is the dominant role of an aberrant activation of the pro-oncogenic PIK3/Akt/mTOR pathway. This was corroborated by the two WES-examined series, which highlighted the potential genomic driver function of *PIK3CA* alterations mostly in HPV-positive VSCC but occasionally also in HPV-negative cases. In addition, it must be remembered that not only direct mutations of signaling cascade members, but also indirect regulatory effects such as the abrogation of cellular mTOR degradation via a loss of function of FBXW7 may confer aberrant PIK3/Akt/mTOR signaling activity. The same is true for copy number alterations. Han et al. recently reported a high incidence of *PIK3CA* copy number gains, either independent of or concurrent with *PIK3CA* mutations in VSCC, reaching a total *PIK3CA* alteration-rate of 60% in their HPV-positive cohort [3].

Besides confirming mutations in other genes (such as *SYNE1*, *SYNE2*, *NSD1*, *KMT2D*, *CDKN2A*), that have already been detected in previous genomic VSCC analyses, the highlight of the work by Prieske et al. is the identification of eight "*new-comer mutations*", not previously described in the context of VSCC [2]. *NBPF1* is one of these "new-comers" showing a missense mutation independently of the HPV status in 20.6% of VSCC. The most intriguing aspect of NBPF1 is that its tumor-suppressive attributes (i.e. inhibition of invasion and promotion of apoptosis) are achieved through direct suppression of the PIK3/Akt/mTOR signaling pathway as evidenced in cervical squamous cancer cells [6]. The influence of NBPF1 on this pathway was further affirmed by its revealed inhibitory effect on the Akt-p53-cyclin D signaling in cutaneous squamous cancer cells [7]. Furthermore as yet undescribed genomic alterations in VSCC are those affecting the tumor suppressor gene *TSC2*. Loss of adequate TSC2 function also leads to hyperactivation of mTOR signaling

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[8]. All this reinforces the notion that cell growth- and survival-promoting PIK3/Akt/mTOR signaling displays a master oncogenic function in VSCC and could constitute an attractive pathway for targeted therapeutical approaches, especially in HPV-related VSCC.

It was astonishing to find *POLE* and *MSH6* mutations among the eight newly detected genomic aberrations in VSCC with a frequency of 9% each. It is unclear, whether the affected VSCC (18%) should also represent ultramutated or hypermutated tumors in analogy to the subtypes in endometrial cancer defined on the molecular basis of TCGA [9]. This is naturally coupled with the question whether these cancers should be ideal candidates for immune checkpoint inhibitor therapy. Data on the crude number of mutations per tumor as provided by Prieske et al. show that one *POLE* mutated cancer exhibiting 2127 mutations and should be classified as ultramutated and another one with 362 mutations as hypermutated. While the three patients with *POLE*-mutated cancers so far continue to be recurrence-free, it remains speculative whether these cancers share a prognosis that is just as overwhelming as that of *POLE*-affected endometrial cancers. In the *MSH6*-mutated and thus mismatch repair-deficient VSCC the mutational load is, however, in the average range of all the other investigated cancers and cannot be regarded as hypermutated. It should be underlined that in general the tumor mutational burden (TMB) of 2.2 mutations per Mb is low in VSCC irrespective of the HPV status, and VSCC can therefore not be assumed to be generally predestined for a single-agent immune checkpoint inhibitor treatment.

Comparison of the existing knowledge of the molecular background of the HPV-related subgroup of VSCC with the TCGA results from the SCC of the uterine cervix (assumed to be 99% HPV-associated) is worthwhile, because of the highly related pathogenesis [10]. Although in cervical SCC, *TP53* mutations and HPV positivity were not mutually exclusive, their occurrence was nevertheless very uncommon (3.5%). The most meaningful overlap in genomic alterations between both HPV-related tumor entities was the high frequency of mutations in *PIK3CA* and *FBXW7*, whereas alterations in *MAPK1* and in *EP300*, which were frequently observed in cervical SCC, were completely absent or strongly under-represented in VSCC. Nonetheless, the TCGA data from cervical SCC indirectly, but unequivocally underscore the HPV- and *TP53*-based "two-disease theory" of VSCC.

Despite the great endeavors undertaken by Prieske and coworkers, we are only at the beginning of the journey called "comprehensive molecular characterization of VSCC." The major obstacle to actually drawing reliable conclusions and defining clinically meaningful molecular subgroups, as for other cancers, is the small size of samples investigated by adequate techniques to date. That limited knowledge currently makes it difficult to estimate the real pathogenicity and the clinical relevance of non-synonymous alterations detected in VSCC and to understand, which alteration is the main oncogenic driver and what exactly is the contextual role of the passenger mutations. In addition, we have more lessons to learn in VSCC. While expanding our classical genomic knowledge we should concomitantly acquire new insights into the non-coding genome and into the epigenetics and furthermore conduct more in depth investigations on copy number gains and losses on the level of individual genes. In this way the network of the oncogenic processes in VSCC will be rigorously disclosed, while keeping in mind that the urgent objective is to unmask targets for innovative treatment strategies. The results presented here aim to provide a strong motivation to continue this research in a women's cancer that shows an increasing incidence, but that for unknown reasons was forgotten, overlooked, neglected or ignored. Whatever the case may be, we now need to make up for lost time!

## Declaration of Competing Interest

The author declares that he has no conflict of interests.

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