EGFR and αvβ6 as Promising Targets for Molecular Imaging of Cutaneous and Mucosal Squamous Cell Carcinoma of the Head and Neck Region

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Supplementary Materials

Table S1. Antibodies and reagents used.

| Target | Catalog Number | Source | Species | Monoclonal/Polyclonal | Antigen Retrieval | Dilution (μg/mL) |
|--------|----------------|--------|---------|-----------------------|-------------------|-----------------|
| αvβ6   | 6.2A1          | Biogen, Inc., Cambridge, MA, USA | Mouse | Monoclonal          | 0.4% pepsin (S3002 Agilent) 37 °C for 10 min. | 0.5 |
| β3     | #13166         | Cell Signaling Technology, Inc., Danvers, MA, USA | Mouse | Monoclonal          | Target retrieval solution, low pH (K8005 Agilent) 95 °C for 10 min with PT Link (Agilent). | 100 |
| CEA    | SC-23928       | Santa Cruz Biotechnology, Inc., Dallas, TX, USA | Mouse | Monoclonal          | Target retrieval solution, low pH (K8005 Agilent) 95 °C for 10 min with PT Link (Agilent). | 0.2 |
| EGFR   | M7239          | Agilent Technologies, Inc., Santa Clara, CA, USA | Mouse | Monoclonal          | 0.4% pepsin (S3002 Agilent) 37 °C for 10 min. | 1.4 |
| EpCAM  | MA5-12436      | Thermo Fisher Scientific, Inc., Waltham, MA, USA | Mouse | Monoclonal          | 0.1% trypsin (T7409 Sigma Aldrich) 37°C for 30 min. | 0.3 |
| uPAR   | ATN617         | Kind gift of A.P. Mazar | Mouse | Monoclonal          | Target retrieval solution, low pH (K8005 Agilent) 95 °C for 10 min with PT Link (Agilent). | 1.2 |
| VEGF-A | RB-9031-P0-A   | Thermo Fisher Scientific, Inc., Waltham, MA, USA | Rabbit | Polyclonal         | Target retrieval solution, low pH (K8005 Agilent) 95 °C for 10 min with PT Link (Agilent). | 0.3 |
| Secondary Antibodies | anti-mouse | K4001 | Agilent Technologies, Inc., Santa Clara, CA, USA | – | – | – | Ready-to-use |
|----------------------|------------|-------|-------------------------------------------------|----|----|----|------------|
| anti-rabbit          | K4003      |       | Agilent Technologies, Inc., Santa Clara, CA, USA | – | – | – | Ready-to-use |
Figure S1. Evaluating the suitability of targets for FGS by the new TBS method is done as described: (A) A pathologist marked the tumour borders on H&E stainings. (B) These borders were evaluated using immunohistochemical staining for the difference in intensity between the tumour area and surrounding tissue and the percentage of the border that stained with this intensity difference. The TBS is a product of the intensity and the group number the percentage score fits in (0 < 5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, 4 > 75%). In this case the intensity difference is 3 and the percentage group is 4 (>75% of the border stains with this percentage difference) resulting in a TBS of 12. (C) In this case the intensity difference is 3 but less than 25% of the border stains with this difference (percentage group 1), resulting in a TBS of 3. (D) The TBS method does not discriminate between tumour or stroma cell staining. In this case, the tumour staining is weak, but the stroma staining along the border still allows for the differentiation between tumour and normal tissue. The intensity difference is 2, and the percentage group is 4, resulting in a TBS of 8. All images are taken at 2× magnification. Black line: border between tumour and surrounding tissue, dark green line: intensity difference of 3, light green line: intensity difference of 2, red line: intensity difference of 0. T: tumour, TBS: tumour-border score, FGS: molecular fluorescence-guided surgery, H&E: hematoxylin & eosin staining.