ABSTRACTS

European Head & Neck Society

January 28–29, 2022
Essen, Germany

3rd INTERNATIONAL SYMPOSIUM on Tumor-Host Interaction in Head and Neck Cancer

11th Symposium of the Working Group Oncology

under the auspices of the

German Society of Oto-Rhino-Laryngology, Head and Neck Surgery

www.headandneck-symposium.de

Starten Sie mit KEYTRUDA®, unabhängig von einer Platin-basierten Vortherapie:

M/uR HNSCC 1L (CPS ≥ 1)

ÜBERLEBENSERWARTUNG NEU DEFINIERT

KEYTRUDA® ist als **Monotherapie** oder in **Kombination**

mit Platin- und 5-FU-Chemotherapie zur Erstlinienbehandlung des M/uR HNSCC bei Erwachsenen mit PD-L1-exprimierenden Tumoren (CPS ≥ 1) angezeigt

www.keytruda.de

KEYTRUDA®
Pembrolizumab, MSD

5-FU = 5-Fluorouracil | M/uR = metastasierend oder nicht resezierbar rezidivierend (metastatic or unresectable recurrent) | HNSCC = Plattenepithelkarzinom der Kopf-Hals-Region (head and neck squamous cell carcinoma) | PD-L1 = programmierter Zelltod-Ligand 1 (programmed cell death ligand 1) | CPS = Combined Positive Score

MSD

KEYTRUDA®

KEYTRUDA® 25 mg/ml Konzentrat zur Herstellung einer Infusionslösung
Wirkstoff: Pembrolizumab **Zus.:** *Arzneil. wirks. Bestandt.:* 1 Durchstechfl. (4 ml) enth. 100 mg Pembrolizumab. 1 ml Konz. enth. 25 mg Pembrolizumab. *Sonst. Bestandt.:* L-Histidin, L-Histidinhydrochlorid-Monohydrat, Sucrose, Polysorbat 80 (E 433), Wasser für Injekt.-zwecke. **Anw.:** Als Monother. zur Behandl. d. fortgeschrittenen (nicht resezierbaren od. metastasierenden) Melanoms b. Erw. Als Monother. zur adjuvanten Behandl. des Melanoms im Tumorstadium III m. Lymphknotenbeteiligung. nach vollständ. Resektion bei Erw. Als Monother. zur Erstlinienbehandl. d. metastasierenden nicht-kleinzelligen Lungenkarzinoms (NSCLC) m. PD-L1 exprimierenden Tumoren (Tumor Proportion Score [TPS] ≥ 50 %) ohne EGFR- od. ALK-pos. Tumormutationen b. Erw. In Komb. m. Pemetrexed u. Platin-Chemotherapie zur Erstlinienbehandl. d. metastasierenden nicht-plattenepithelialen NSCLC ohne EGFR- od. ALK-pos. Tumormutationen bei Erw. In Komb. m. Carboplatin u. entweder Paclitaxel od. nab-Paclitaxel zur Erstlinienbehandl. d. metastasierenden plattenepithelialen NSCLC bei Erw. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden NSCLC m. PD-L1 exprimierenden Tumoren (TPS ≥ 1 %) nach vorheriger Chemother. b. Erw. Pat. m. EGFR- od. ALK-pos. Tumormutationen sollten vor Ther. ebenfalls eine auf diese Mutationen zielgericht. Ther. erhalten haben. Als Monother. zur Behandl. d. rezidivierenden od. refraktären klassischen Hodgkin-Lymphoms (HL) b. Kdrn. u. Jugendl. ab 3 Jahren u. Erw. nach Versagen einer autologen Stammzelltransplantation (auto-SZT) od. nach mind. 2 vorangegang. Ther., wenn eine auto-SZT nicht in Frage kommt. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden Urothelkarzinoms nach vorheriger Platin-basierter Ther. b. Erw. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden Urothelkarzinoms bei Erw., die nicht für e. Cisplatin-basierte Ther. geeignet sind u. deren Tumoren PD-L1 m. einem kombinierten positiven Score (CPS) ≥ 10 exprimieren. Als Monother. od. in Komb. m. Platin- u. 5-Fluorouracil(5-FU)-Chemother. zur Erstlinienbehandl. d. metastasierenden od. nicht resezierbaren rezidivierenden Plattenepithelkarzinoms der Kopf-Hals-Region (HNSCC) b. Erw. m. PD-L1-exprimierenden Tumoren (CPS ≥ 1). Als Monother. zur Behandl. d. rezidivierenden od. metastasierenden HNSCC m. PD-L1-exprimierenden Tumoren (TPS ≥ 50 %) und einem Fortschreiten der Krebserkrank. während od. nach vorheriger Platin-basierter Ther. b. Erw. In Komb. m. Axitinib zur Erstlinienbehandl. d. fortgeschrittenen Nierenzellkarzinoms (RCC) b. Erw. Als Monother. zur Erstlinienbehandl. d. metastasierenden Kolorektalkarzinoms (CRC) b. Tumoren m. hochfrequenter Mikrosatelliten-Instabilität (MSI-H) od. m. e. Mismatch-Reparatur-Defizienz (dMMR) b. Erw. In Komb. m. e. Platin- u. Fluoropyrimidin-basierten Chemother. zur Erstlinienbehandl. d. lokal fortgeschrittenen nicht resezierbaren od. metastasierenden Ösophaguskarzinoms od. d. HER2-negativen Adenokarzinoms d. gastroösophagealen Übergangs b. Erw. mit PD-L1-exprimierenden Tumoren (CPS ≥ 10). **Gegenanz.:** Überempf.-keit gg. d. Wirkstoff od. e. d. sonst. Bestandt. **Vorsicht bei:** Schwerer Einschränk. d. Nierenfunkt.; moderater od. schwerer Einschränk. d. Leberfunkt. Melanom d. Auges. Anamnest. bek. immunvermittelte Myokarditis. Behandl. nach Risikoabwägung b. Pat. m.: aktiven ZNS-Metastasen; ECOG-Performance-Status ≥ 2; HIV-, HBV- od. HCV-Infekt.; aktiven, system. Autoimmunerkrank.; interstit. Lungenkrankh.; einer früheren Pneumonitis, d. system. Kortikoidbehandl. erforderte; schwerer Überempf.-keit gg. e. and. monoklonalen Antikörper in d. Anamnese; laufender Ther. m. Immunsuppressiva; schweren immunvermittelten Nebenw. unter Ipilimumab in d. Anamnese (jegliche Grad 4 od. Grad 3 Toxizität), d. eine Kortikosteroid-Behandl. über mehr als 12 Wo. erforderte (mehr als 10 mg/Tag Prednison od. Äquivalent in entspr. Dosierung); aktiv. Infekt.-erkrank.; Pat., d. unter vorhergeh. Krebsbehandl. m. immunstimulierenden Arzneim. schwere od. lebensbedrohl. Nebenw. d. Haut hatten; Pat., d. ein solides Organtransplantat empfangen haben; Pat. m. allo-HSZT in Krankengesch. Hinw. zu Schwangersch./Stillzeit beachten. Unter den immunvermittelten Nebenw. waren schwere Fälle u. Todesfälle. *Zusätzl. bei HNSCC:* Pat. m. vorheriger Strahlenther. *Zusätzl. bei klass. HL:* Pat. ≥ 65 J. Bei allo-HSZT bei klassischem HL nach Ther. m. Pembrolizumab sorgfältige Nutzen-Risiko-Abwägung (GVHD u. schwere Lebervenenverschlusskrankheit als Komplikat. beobachtet). *Zusätzl. bei reseziertem Stadium-III-Melanom, MSI-H- od. dMMR-CRC, fortgeschrittenem RCC, Erstlinienbehandl. bei NSCLC od. Ösophaguskarzinom u. Erstlinienbehandl. bei HNSCC:* Pat. ≥ 75 J. *Zusätzl. bei Urothelkarzinom n. vorh. Platin-basierter Ther.:* Pat. m. schlechterer Prognose u./od. aggressiv. Krankheitsverl. *Zusätzl. bei Pat. m. Urothelkarzinom, d. nicht für e. Cisplatin-Ther. geeignet sind u. deren Tumoren PD-L1 m. CPS ≥ 10 exprimieren:* Pat. d. für Komb.-chemother. m. Carboplatin geeignet sind. *Bei Erstlinienbehandl. von NSCLC od. HNSCC m. PD-L1 exprimierenden Tumoren:* Nutzen u. Risiko e. Komb. m. Chemother. im Vgl. zu Pembrolizumab Monother. abwägen. **Nebenw.:** *Monother.: Sehr häufig:* Anämie. Hypothyreose. Vermind. Appetit. Kopfschm. Dyspnoe; Husten. Diarrhö; Abdominalschm.; Übelk.; Erbr.; Obstipat. Hautausschl.; Pruritus. Muskuloskelett. Schm.; Arthralgie. Müdigk./Erschöpf.; Asthenie; Ödeme; Fieber. *Häufig:* Pneumonie. Thrombozytopenie; Neutropenie; Lymphopenie. Infusionsbed. Reakt. Hyperthyreose; Thyreoiditis. Hyponatriämie; Hypokaliämie; Hypokalzämie. Schlaflosigk. Schwindelgef.; periph. Neuropathie; Lethargie; Geschmacksstör. Trock. Augen. Kardiale Arrhythmie (einschl. Vorhofflimmern). Hypertonie. Pneumonitis. Kolitis; Mundtrockenh. Schwere Hautreakt.; Erythem; Dermatitis; trock. Haut; Vitiligo; Ekzem; Alopezie; akneiforme Dermatitis. Schm. in d. Extremitäten; Myositis; Arthritis. Grippeähnl. Erkrank.; Schüttelfrost. AST erhöht; ALT erhöht; Hyperkalzämie; alkal. Phosphatase im Blut erhöht; Bilirubin im Blut erhöht; Kreatinin im Blut erhöht. *Gelegentl.:* Leukopenie; Eosinophilie. Sarkoidose. Nebennereninsuff.; Hypophysitis. Typ-1-Diabetes-mellitus. Epilepsie. Uveitis. Myokarditis; Perikarderguss; Perikarditis. Pankreatitis; Gastritis; gastrointestinale Ulzeration. Hepatitis. Psoriasis; lichenoide Keratose; Papeln; Änd. d. Haarfarbe. Tendosynovitis. Nephritis. Amylase erhöht. *Selten:* Immunthrombozytopenie; hämolyt. Anämie; isolierte aplast. Anämie; hämophagozytische Lymphohistiozytose. Enzephalitis; Guillain-Barré-Syndrom; Myelitis; Myasthenie-Syndrom; Meningitis (aseptisch). Vogt-Koyanagi-Harada-Syndrom. Vaskulitis. Dünndarmperforation. Sklerosierende Cholangitis. TEN; SJS; Erythema nodosum. Sjögren-Syndrom. *Nicht bekannt:* Abstoßung e. soliden Organtransplantats. *Zusätzl.:* Hinw. zu Abw. bei Laborwerten beachten. *B. Komb. m. Chemother.: Sehr häufig:* Pneumonie. Anämie; Neutropenie; Thrombozytopenie. Hypothyreose. Hyponatriämie; Hypokaliämie; vermind. Appetit. Schlaflosigkeit. Schwindelgef.; periphere Neuropathie; Kopfschm. Dyspnoe; Husten. Übelk.; Diarrhö; Erbr.; Abdominalschm.; Obstipat. Hautausschl.; Alopezie; Pruritus. Muskuloskelett. Schm.; Arthralgie. Müdigk./Erschöpf.; Asthenie; Fieber; Ödeme. Kreatinin im Blut erhöht. *Häufig:* Febrile Neutropenie; Leukopenie; Lymphopenie. Infusionsbed. Reakt. Hyperthyreose. Hypokalzämie. Geschmacksstör.; Lethargie. Trock. Augen. Kardiale Arrhythmie (einschl. Vorhofflimmern). Vaskulitis; Hypertonie. Pneumonitis. Kolitis; Mundtrockenh.; Gastritis. Hepatitis. Schwere Hautreakt.; trock. Haut; Erythem; Dermatitis. Myositis; Schm. in d. Extremitäten; Arthritis. Akutes Nierenvers. Grippeähnl. Erkrank.; Schüttelfrost. AST erhöht; Hyperkalzämie; ALT erhöht; alkal. Phosphatase im Blut erhöht; Bilirubin im Blut erhöht. *Gelegentl.:* Eosinophilie. Hypophysitis; Nebenniereninsuff.; Thyreoiditis. Typ-1-Diabetes-mellitus. Enzephalitis; Epilepsie. Myokarditis; Perikarderguss. Pankreatitis; gastrointestinale Ulzeration. Psoriasis; Vitiligo; Ekzem; akneiforme Dermatitis; Papeln; lichenoide Keratose. Tendosynovitis. Nephritis. Amylase erhöht. *Selten:* Guillain-Barré-Syndrom. Perikarditis. Sklerosierende Cholangitis. Änd. d. Haarfarbe. Sjögren-Syndrom. *Zusätzl.:* Hinw. zu Abw. bei Laborwerten beachten. *B. Komb. m. Axitinib: Sehr häufig:* Hyperthyreose; Hypothyreose. Vermind. Appetit. Kopfschm.; Geschmacksstör. Hypertonie. Dyspnoe; Husten; Dysphonie. Diarrhö; Abdominalschm.; Übelk.; Erbr.; Obstipat. Palmar-plantares Erythrodysästhesie-Syndr.; Hautausschl.; Pruritus. Muskuloskelett. Schm.; Arthralgie; Schm. in d. Extremitäten. Müdigk./Erschöpf.; Asthenie; Fieber. ALT erhöht; AST erhöht; Kreatinin im Blut erhöht. *Häufig:* Pneumonie. Anämie; Neutropenie; Leukopenie; Thrombozytopenie. Infusionsbed. Reakt. Hypophysitis; Thyreoiditis; Nebenniereninsuff. Hypokaliämie; Hyponatriämie; Hypokalzämie. Schlaflosigk. Schwindelgef.; Lethargie; periphere Neuropathie. Trock. Augen. Kardiale Arrhythmie (einschl. Vorhofflimmern). Pneumonitis. Kolitis; Mundtrockenh.; Gastritis. Hepatitis. Schwere Hautreakt.; akneiforme Dermatitis; Dermatitis; trock. Haut; Alopezie; Ekzem; Erythem. Myositis; Arthritis; Tendosynovitis. Akutes Nierenvers.; Nephritis. Ödeme; grippähnl. Erkrank.; Schüttelfrost. Alkal. Phosphatase im Blut erhöht; Hyperkalzämie; Bilirubin im Blut erhöht. *Gelegentl.:* Lymphopenie; Eosinophilie. Typ-1-Diabetes-mellitus. Myasthenie-Syndrom. Uveitis. Myokarditis. Pankreatitis; gastrointestinale Ulzeration. Änd. d. Haarfarbe; lichenoide Keratose; Papeln; Psoriasis; Vitiligo. Sjögren-Syndrom. Amylase erhöht. *Zusätzl.:* Hinw. zu Abw. bei Laborwerten beachten. **Warnhinw.:** Nicht schütteln. **Hinw.:** Falls im Anwendungsgebiet angegeben, Untersuch. der PD-L1-Tumor-Expression mittels eines validierten Tests. Untersuch. des MSI-H/dMMR-Tumorstatus mittels eines validierten Tests b. Pat. m. CRC. Zuverlässige Verhütungsmethode b. Frauen im gebärf. Alter währ. Behandl. u. bis min. 4 Mon. nach letzter Dosis. **Verschreibungspflichtig.** Stand: 06/2021a

Bitte lesen Sie vor Verordnung von KEYTRUDA® die Fachinformation!

Pharmazeutischer Unternehmer:
Merck Sharp & Dohme B.V.
Waarderweg 39
2031 BN Haarlem
Niederlande

Lokaler Ansprechpartner:
MSD Sharp & Dohme GmbH
Lindenplatz 1, 85540 Haar

MSD Infocenter
Tel. 0800 673 58 38
Fax 0800 673 673 329
E-Mail infocenter@msd.de

RCN: 000018752-DE
DE-OHN-00157

3rd International Symposium on Tumor-Host Interaction in Head and Neck Cancer

in conjunction with the

11th Symposium of the Working Group Oncology

Editor: Prof. Dr. med. Stephan Lang
ISBN: 978-3-948023-21-8

OPDIVO® (nivolumab)

Bewährtem vertrauen. Perspektive geben.

OPDIVO® – der PD-1-Antikörper mit breiter Zulassung:*,1

- NSCLC[a,b]
- Melanom[a,b]
- Nierenzellkarzinom[a,b]
- Adenokarzinome des oberen GI-Trakts (EAC, GEJC, GC)[b]
- Ösophaguskarzinom[a]
- Kolorektales Karzinom[b]
- Malignes Pleuramesotheliom[b]
- Kopf-Hals-Tumore[a]
- Urothelkarzinom[a]
- Klassisches Hodgkin-Lymphom[a]

6 Jahre OPDIVO® in Deutschland#

Bristol Myers Squibb™

bms-onkologie.de

OPDIVO® 10 mg/ml Konzentrat z. Herst. e. Infusionslösung. **Wirkstoff:** Nivolumab. **Sonst. Bestandteile:** Natriumcitratdihydrat; Natriumchlorid; Mannitol; Pentetsäure; Polysorbat 80; Natriumhydroxid; Salzsäure u. Wasser f. Injektionszwecke. **YERVOY®** 5 mg/ml Konzentrat z. Herst. e. Infusionslösung. **Wirkstoff:** Ipilimumab. **Sonst. Bestandteile:** Trometamolhydrochlorid; Natriumchlorid; Mannitol; Pentetsäure; Polysorbat 80; Natriumhydroxid; Salzsäure u. Wasser f. Injektionszwecke. **Anw. OPDIVO®/ YERVOY® in Komb. mit Ipilimumab/ Nivolumab:** Behandl. d. fortgeschritt. (nicht resezierb. oder met.) Melanoms b. Erw.. Im Vergl. z. Nivolumab Monother. wurde in der Komb. Nivolumab mit Ipilimumab nur b. Patienten mit niedr. Tumor PD-L1-Expression ein Anstieg d. PFS u. OS gezeigt. Erstlinienther. d. fortgeschritt. RCC b. Erw. mit intermediärem/ungünstigem Risikoprofil. Mit 2 Zyklen Pt basierter CTx. f. die Erstlinienther. d. met. NSCLC b. Erw., deren Tumoren keine sensitivierende EGFR-Mutation od. ALK-Translokation aufweisen. Erstlinienther. d. nicht-resezierb. malignen Pleuramesothelioms bei Erw.. Behandl. d. met. dMMR- oder MSI-H-CRC bei Erw. nach vorheriger fluoropyrimidinbasierter Kombinations-CTx. **OPDIVO® in Komb. mit Chemotherapie:** In Komb. mit fluoropyrimidin- u. Pt basierter Kombinations-CTx für die Erstlinienbehandl. d. HER2-negat. fortgeschritt. od. met. Adenokarzinome d. Magens, d. gastroösophagealen Übergangs od. d. Ösophagus b. Erw., deren Tumoren PD-L1 (CPS ≥ 5) exprimieren. **OPDIVO® in Komb. mit Cabozantinib:** Erstlinienther. d. fortgeschritt. RCC b. Erw.. **Gegenanz.:** Überempf.-keit gg. d. Wirkstoff od. sonst. Bestandt.. **Nebenwirk.: Komb. Nivolumab mit Ipilimumab und/oder Chemotherapie: Sehr häufig:** Infekt. d. oberen Atemwege; Hypothyreose; Hyperthyreose; vermind. Appetit; periph. Neuropathie; Kopfschm.; Schwindelgef.; Hypertonie; Dyspnoe; Husten; Kolitis; Diarrhö; Stomatitis; Erbr.; Übelk.; Obstipation; Bauchschm.; Palmar-plant. Erythrodysaesthesiesyndr.; Hautausschl.; Pruritus; trock. Haut; Muskel- u. Skelettschm.; Arthralgie; Fatigue; Pyrexie; Ödeme (einschl. periph. Ö.); Anstieg AST, ALT, Gesamtbilirubin, alkal. Phosphatase, Lipase, Amylase, Kreatinin; Hyperglykämie; Hypoglykämie; Lymphopenie; Leukopenie; Neutropenie; Thrombozytopenie; Anämie; Hyperkalziämie; Hypokalzämie; Hyperkaliämie; Hypokaliämie; Hypomagnesiämie; Hypernatriämie; Hyponatriämie. **Häufig:** Pneumonie; Bronchitis; Konjunktivitis; febrile Neutropenie; Eosinophilie; Infusionsbed. Reakt.; Hypersensib.; Nebennierenninsuff.; Hypophyseninsuff.; Hypophysitis; Thyroiditis; Diabetes mell.; Dehydr.; Hypoalbuminämie; Hypophosphatämie; Parästhesie; Uveitis; verschwom. Sehen; trock. Augen; Tachykardie; Thrombose; Pneumonitis; Pleuraerguss; Lungenembolie; Pankreatitis; trock. Mund; Gastritis; Hepatitis; Hauthyperpigmentier.; Vitiligo; Erythem; Alopezie; Urtikaria; Arthritis; Muskelspasmen; musk. Schwäche; Nierenvers. (einschl. akutem N.); Schm.; Schm. in d. Brust; Schüttelfrost; Hypermagnesiämie; Gew.-Verlust; Anstieg TSH. **Gelegentl.:** Asept. Meningitis; Sarkoidose; diab. Ketoazidose; Hypoparathyreoidismus; metabol. Azidose; Guillain-Barré-Syndr.; Polyneuropathie; Neuritis; Peroneuslähm.; autoimm. Neuropathie (einschl. Gesichtsnerv- u. Abduzensparese); Myasthenia gravis; Enzephalitis; Arrhythmie (einschl. ventrik. A.); Bradykardie; Vorhofflimm.; Myokarditis; Episkleritis; Darmperfor.; Duodenitis; Psoriasis; Stevens-Johnson-Syndr.; Erythema multiforme; Spondyloarthropathie; Sjögren-Syndr.; Myopathie; Polymyalgia rheumatica; Myositis (einschl. Polym.); Rhabdomyolyse; tubulointerstit. Nephritis; Nephritis; nicht-infektiöse Zystitis; Anstieg Gammaglutamyltransferase. **Selten:** Tox. epiderm. Nekrolyse. **Nicht bekannt:** Hämophagozyt. Lymphohistiozytose; Abstoß. solides Organtransplantat; Hyperparathyreoidismus; Tumorlyse-Syndr.; Vogt-Koyanagi-Harada-Syndr.; perikard. Erkrank.; Lichen sclerosus; and. Lichenerkank.. **Komb. Nivolumab mit Cabozantinib: Sehr häufig:** Infekt. d. oberen Atemwege; Hypothyreose; Hyperthyreose; vermind. Appetit; Dysgeusie; Schwindelgef.; Kopfschm.; Hypertonie; Dysphonie; Dyspnoe; Husten; Diarrhö; Erbr.; Übelk.; Obstipation; Stomatitis; Bauchschm.; Dyspepsie; Palmar-plant. Erythrodysaesthesiesyndr.; Hautausschl.; Pruritus; Muskel- u. Skelettschm.; Arthralgie; Muskelspasmen; Proteinurie; Fatigue; Pyrexie; Ödeme; Anämie; Thrombozytopenie; Leukopenie; Lymphopenie; Neutropenie; Anstieg alkal. Phosphatase, ALT, AST, Gesamtbilirubin, Kreatinin, Amylase, Lipase; Hypokaliämie; Hypomagnesiämie; Hyponatriämie; Hypokalzämie; Hyperkalzämie; Hypoglykämie; Hypophosphatämie; Hyperglykämie; Hyperkaliämie; Hypermagnesiämie; Hypernatriämie; Gew.-Verlust. **Häufig:** Pneumonie; Eosinophilie; Hypersensib. (einschl. anaphylaktische Reakt.); Nebennierenninsuff.; Dehydr.; periph. Neuropathie; Tinnitus; trock. Augen; verschwom. Sehen; Vorhofflimm.; Tachykard.; Thrombose; Pneumonitis; Lungenembolie; Pleuraerguss; Epistaxis; Kolitis; Gastritis; Mundschm.; trock. Mund; Hämorrhoiden; Hepatitis; Alopezie; trock. Haut; Erythem; Änd. d. Haarfarbe; Arthritis; Nierenvers.; akute Nierenschädig.; Schm.; Schm. in d. Brust; Anstieg Cholesterin im Blut; Hypertriglyzeridämie. **Gelegentl.:** Infusionsbed. Überempfindl.-reakt.; Hypophysitis; Thyroiditis; autoimm. Enzephalitis; Guillain-Barré-Syndr.; myasthenes Syndr.; Uveitis; Myokarditis; Pankreatitis; Dünndarmperfor.; Glossodynie; Psoriasis; Urtikaria; Myopathie; Osteonekrose d. Kiefers; Fistel; Nephritis. **Selten:** Nicht-infektiöse Zystitis. **Nicht bekannt:** Lichen sclerosus; and. Lichenerkrank..

Weitere Hinweise siehe jeweilige Fachinformation. Verschreibungspflichtig. Pharmazeutischer Unternehmer: Bristol-Myers Squibb Pharma EEIG; Plaza 254; Blanchardstown Corporate Park 2; Dublin 15; D15 T867; Irland. Stand d. Textes: V13

OPDIVO® 10 mg/ml Konzentrat z. Herst. e. Infusionslösung. **Wirkstoff:** Nivolumab. **Sonst. Bestandteile:** Natriumcitratdihydrat, Natriumchlorid, Mannitol, Pentetsäure, Polysorbat 80, Natriumhydroxid, Salzsäure u. Wasser f. Injektionszwecke. **Anw.:** Als Monother. b. Erw. f. d. Behandl. d. fortgeschritt. (nicht resezierb. od. met.) Melanoms. Als Monother. b. Erw. zur adjuv. Behandl. d. Melanoms mit Lymphknotenbeteilig. od. Metastasierg. nach vollst. Resektion. Als Monother. zur Behandl. d. lokal fortgeschritt. od. met. NSCLC nach vorheriger CTx b. Erw.. Als Monother. b. Erw. zur Behandl. d. fortgeschritt. RCC nach Vorther.. Als Monother. zur Behandl. d. rezidiv. od. refrakt. cHL b. Erw. nach ASCT u. Behandl. m. Brentuximab Vedotin. Als Monother. zur Behandl. d. rezidiv. od. met. Plattenepithelkarzinoms d. Kopf-Hals-Bereichs b. Erw. mit Progress. während od. nach Pt-basierter Ther.. Als Monother. zur Behandl. d. lokal fortgeschritt. nicht resezierb. od. met. Urothelkarzinoms b. Erw. nach Versagen vorheriger Pt-haltiger Ther.. Als Monother. zur Behandl. d. nicht resezierb. fortgeschritt., rezidiv. od. met. Plattenepithelkarzinoms d. Ösophagus b. Erw. nach vorheriger fluoropyrimidin- u. Pt-basierter Komb.-CTx. Als Monother. zur adjuv. Behandl. d. Karzinome d. Ösophagus od. d. gastroösophag. Übergangs b. Erw. mit patholog. Resterkr. nach vorheriger neoadjuv. Chemoradiother.. **Gegenanz.:** Überempf.-keit gg. d. Wirkstoff od. sonst. Bestandt.. **Nebenwirk.: Sehr häufig:** Infekt. d. oberen Atemwege; vermind. Appetit; Kopfschm.; Dyspnoe; Husten; Diarrhö; Erbr.; Übelk.; Bauchschm.; Obstipation; Hautausschl.; Pruritus; Muskel- u. Skelettschm.; Arthralgie; Fatigue; Pyrexie; Ödeme; Lymphopenie; Hyperglykämie; Anämie; Anstieg AST; Hyponatriämie; Hypoalbuminämie; Anstieg alkal. Phosphatase, Kreatinin, ALT, Lipase; Hyperkaliämie; Anstieg Amylase; Hypokalziämie; Leukopenie; Hypomagnesiämie; Neutropenie; Thrombozytopenie; Hypokaliämie; Hypoglykämie; Hyperkalziämie. **Häufig:** Pneumonie; Bronchitis; Infusionsbed. Reakt.; Hypersensib. (einschl. anaphylakt. Reakt.); Hypothyreose; Hyperthyreose; Thyroiditis; Dehydr.; periph. Neuropathie; Schwindelgef.; verschwomm. Sehen; trock. Augen; Tachykard.; Vorhofflimm.; Hypertonie; Pneumonitis; Pleuraerguss; Kolitis; Stomatitis; trock. Mund; Vitiligo; trock. Haut; Erythem; Alopezie; Arthritis; Nierenvers. (einschl. akutem N.); Schm.; Schm. in d. Brust; Anstieg Gesamtbilirubin; Hypermagnesiämie; Hypernatriämie; Gew.-Verlust. **Gelegentl.:** Sarkoidose; Nebennierenninsuff.; Hypophyseninsuff.; Hypophysitis; Diabetes mell.; metabol. Azidose; Polyneuropathie; autoimm. Neuropathie (einschl. Gesichtsnerv- u. Abduzensparese); Uveitis; perikard. Erk.; Arrhythmie (einschl. ventrik. A.); Lungeninfiltr.; Pankreatitis; Gastritis; Hepatitis; Cholestase; Erythema multiforme; Psoriasis; Rosazea; Urtikaria; rheumat. Polymyalgie; tubulointerstit. Nephritis. **Selten:** Asept. Meningitis; Histiozytär nekrotisier. Lymphadenitis (Kikuchi-L.); Eosinophilie; diab. Ketoazidose; Hypoparathyreoidismus; Guillain-Barré-Syndr.; Demyelinisier.; myasthenes Syndr.; Enzephalitis; Myokarditis; Vaskulitis; Zwölffingerdarmgeschw.; tox. epiderm. Nekrolyse; Stevens-Johnson-Syndr.; Sjögren-Syndr.; Myopathie; Myositis (einschl. Polym.); Rhabdomyolyse; nicht-infektiöse Zystitis. **Nicht bekannt:** Hämophagozyt. Lymphohistiozytose; Abstoß. solides Organtransplantat; Tumorlyse-Syndr.; Vogt-Koyanagi-Harada-Syndr.; Lichen sclerosus; and. Lichenerkrank..

Weitere Hinweise siehe Fachinformation. Verschreibungspflichtig. Pharmazeutischer Unternehmer: Bristol-Myers Squibb Pharma EEIG; Plaza 254; Blanchardstown Corporate Park 2; Dublin 15; D15 T867; Irland. Stand: v25.

a Monotherapie b Kombinationstherapie * Details zu den Anwendungsgebieten finden Sie im Pflichttext und in der aktuellen OPDIVO®-Fachinformation.
19.06.2015: EU-Zulassung von Nivolumab bei Erwachsenen für die Behandlung des fortgeschrittenen (nicht resezierbaren oder metastasierten) Melanoms.

1. OPDIVO®-Fachinformation, aktueller Stand

Table of contents

1

HNSCC – immunological and metabolic characterization of different tumor stage and localization

I. Ugele (Regensburg/DE), M. Wehrstein (Regensburg/DE), K. Dettmer-Wilde (Regensburg/DE), P. Oefner (Regensburg/DE), M. Kreutz (Regensburg/DE), C. Bohr (Regensburg/DE)

Questions: In head and neck squamous cell carcinoma (HNSCC) checkpoint-inhibitor therapy is of limited efficacy. Immuno-therapy response depends on T cell infiltration and function, which is affected by the metabolic tumor microenvironment. We hypothesize that tumor localization and tumor stage impact metabolism, thereby immune cell infiltration and function and consequently response to checkpoint blockade.

We investigated the metabolic profile of HNSCC in different localizations (oral cavity, oropharynx, hypopharynx, larynx) and with different tumor stage and correlated it to the immune cell infiltrate.

Methods: Immune infiltrate was analyzed by flow cytometry and the metabolic profile in tissue biopsies as well as interstitial fluid in comparison to corresponding mucosa by mass spectrometry.

Results: We observed general differences in immune infiltration but also tumor stage and localization specific changes. A significant decrease in T cell counts and concomitant increase in myeloid derived suppressor cells was detected, more pronounced in progressed tumors. Furthermore, the expression of T-cell activation markers was reduced in tumor tissue in comparison to corresponding mucosa and was dependent on tumor stage and localization. By investigating 39 central metabolites of glucose metabolism, citric acid cycle and amino acid metabolism, we identified 12 metabolites significantly altered in tumor tissue. Relating metabolite abundance and T cell infiltration and activation revealed significant correlations, e.g. a negative correlation between 2-hydroxy-glutarate levels and T cell counts. Furthermore glutamine levels exerted a significant impact on the expression of CD69.

Conclusion: We show a dependence of stage and localization on tumor metabolism and immune cell infiltrate in HNSCC. Our results provide a prospective for combinations of checkpoint blockade with anti-metabolic drugs to enhance anti-tumoral immune response in HNSCC.

2

Uncoupled biological and chronological ageing of neutrophils in HNSCC promotes tumor progression

B. Uhl (Munich/DE), L. A. Mittmann (Munich/DE), F. Haring (Munich/DE), J. B. Schaubaecher (Munich/DE), R. Hennel (Munich/DE), B. Smiljanov (Munich/DE), G. Zuchtriegel (Munich/DE), M. Canis (Munich/DE), O. Gires (Munich/DE), F. Krombach (Munich/DE), L. Holdt (Munich/DE), S. Brandau (Essen/DE), T. Vogl (Münster/DE), K. Lauber (Munich/DE), C. Reichel (Munich/DE)

Beyond their established role in homeostasis and host defense, neutrophils are increasingly recognized to contribute to the pathogenesis of malignant tumors. Recently, ageing of mature neutrophils in the systemic circulation has been identified to be critical for these immune cells to properly unfold their homeostatic and anti-infectious properties. The role of this process in cancer remains unclear.

Neutrophil trafficking in HNSCC was analyzed in orthotopic (floor of mouth; flow cytometry) and heterotopic (ear; *in vivo* microscopy) mouse models of HNSCC (cell line SCC VII). Adoptive cell transfer and metabolic pulse labeling with BrdU allowed us to determine the relative chronological age of neutrophils in these experiments. Phenotypic and functional properties of neutrophils were characterized by multi-channel flow cytometry and various *in vitro* assays. Intratumoral cytokine levels were assessed by multiplex ELISA.

Here, we demonstrate that molecular signals released during early stages of HNSCC uncouples biological from chronological ageing of neutrophils by accelerating age-related changes in their molecular repertoire. This facilitates the accumulation of these highly reactive immune cells in malignant lesions and endows them with potent pro-tumorigenic functions. In particular, neutrophil elastase released by tumor infiltrating neutrophils stimulates the proliferation of malignant cells, thus promoting tumor progression. As a translational approach, counteracting uncoupled biological ageing of circulating neutrophils by blocking the chemokine receptor CXCR2 effectively suppressed tumor progression.

In conclusion, our experimental data uncover a potent self-sustaining mechanism of HNSCC in fostering pro-tumorigenic phenotypic and functional changes in circulating neutrophils. Interference with this aberrant process might provide a novel, already pharmacologically targetable strategy for HNSCC therapy.

This study was supported by the DFG (SFB 914).

3

Trametinib sensitizes head and neck cancer to immunotherapy via interfering the tumor – host interactions

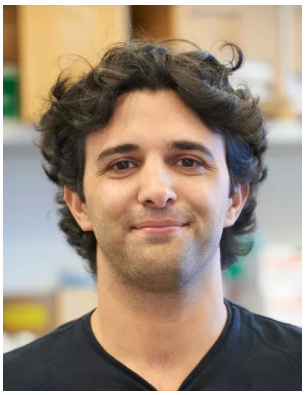
M. Elkabets (Beer-Sheva/IL)

Question: Mutations in genes of the mitogen-activated protein kinases (MAPK) pathway are associated with response to anti-PD-1 in head and neck cancer (HNC), and ~50% of patients expect to show clinical benefit. How can we ehance the response rate and can we achieve a better anti-tumor activity?

Results: Here, we show that pre-treatment with trametinib (a MEK1/2 blocker) followed by anti-PD-1 (αPD-1) supplementation eliminated tumors with an adequate immune memory in the cured mice. Mechanistically we show trametinib delays the progression of MAPK-mutated HNC in preclinical models concomitantly with altering the immune cell heterogeneity in the tumor microenvironment (TME). Specifically, trametinib treatment induced a transient immune-active TME enriched with activated CD8 T cells along with a reduction in the abundance of two CSF-1R+CD11c+ myeloid-derived suppressor cell (MDSC) populations. We further show that treatment with trametinib reduced colony-stimulating factor 1 (CSF-1) expression, determining the abundance of CSF-1R+CD11c+ in the TME. CSF-1 overexpression reduced the immune-active TME and abrogated the tumor elimination effect mediated by the trametinib/ αPD-1 combination therapy. In HNC patients, an immune-active TME defined by a high CD8A to CSF-1 ratio was associated with a clinical benefit derived from αPD-1 therapy supplementation.

Conclusions Our findings provide a strong rationale for (i) interfering tumor-host interaction using MEK1/2 inhibition to enhance response to immunotherapy and (ii) for testing in αPD-1 MAPK-mutated HNC the combination of trametinib and αPD-1 for promoting long-term anti-tumor immunity.

Figure



4

Inhibition of TGFβ carried by tumor-derived exosomes ameliorates angiogenesis and head and neck cancer progression

N. Ludwig (Regensburg/DE), S. Yerneni (Pittsburgh, PA/US), J. Azambuja (Pittsburgh, PA/US), M. Pietrowska (Gliwice/PL), P. Widlak (Gliwice/PL), C. Hinck (Pittsburgh, PA/US), B. Razzo (New York/US), A. Gluszko (Warsaw/PL), M. Szczepański (Warsaw/PL), T. Reichert (Regensburg/DE), A. Hinck (Pittsburgh, PA/US), T. L. Whiteside (Pittsburgh, PA/US)

Introduction: Transforming growth factor β (TGFβ) is a major component of tumor-derived exosomes (TEX) in cancer patients, including those with head and neck squamous cell carcinoma (HNSCC).

Objectives: The goal of this study was to characterize mechanisms utilized by $TGF\beta^+$TEX to promote tumor growth and evaluate their clinical relevance.

Methods: TEX were isolated from HNSCC cell lines by size exclusion chromatography and TGFβ content was evaluated by mass spectrometry and functional bioassays. TEX were co-incubated with endothelial cells (ECs), primary human macrophages and injected *in vivo* using the murine basement membrane extract (BME) plug model. A TGFβ trap (mRER) was used to block TGFβ signaling. The 4-nitroquinoline-1-oxide (4NQO) oral carcinogenesis mouse model was used to track serum-derived $TGF\beta^+$ exosomes. Tumor progression was monitored in 4NQO mice injected with $TGF\beta^+$TEX. TGFβ was quantified in plasma-derived exosomes from HNSCC patients (n=36) or normal donors (NDs; n=10) by functional bioassays or bioprinted microarrays using TGFβ receptor II for capturing.

Results: TEX carried TGFβ and induction of Smad-dependent signaling by TEX stimulated pro-angiogenic functions in ECs and reprogrammed macrophages to the pro-angiogenic M2 phenotype. TEX promoted vascularization ($p<0.001$) in BME plugs which was inhibited by mRER ($p<0.001$). TGFβ levels increased ($p<0.05$) in 4NQO tumor tissue and in serum during carcinogenesis in parallel with increasing numbers of circulating $TGF\beta^+$ exosomes. $TGF\beta^+$TEX injected into 4NQO mice increased vascularization and enhanced TGFβ levels in tumor tissues ($p<0.05$). In contrast to NDs, TGFβ levels were elevated in plasma-derived exosomes from HNSCC patients ($p=0.0026$) and correlated with tumor size ($p=0.0013$).

Conclusion: Signaling by $TGF\beta^+$TEX exerted profound pro-angiogenic activities which were ameliorated by inhibition with mRER. Silencing of TGFβ in TEX emerges as a critical step in suppressing growth of HNSCC.

5

Bi-directional cross-talk of cancer cells and tumor-associated neutrophils triggers innate immune escape

J. Szlachetko (Essen/DE), S. Vollmer (Essen/DE), L. J. Schröder (Essen/DE), M. Busch (Essen/DE), K. Bruderek (Essen/DE), J. Kehrmann (Essen/DE), S. Lang (Essen/DE), N. Dünker (Essen/DE), S. Brandau (Essen/DE)

Introduction: Head and neck carcinomas (HNC) are often associated with bacterial superinfection, high frequency of neutrophils in the tissue and increased metastasis. All these factors are associated with poor prognosis of patients, but their functional interaction is poorly understood.

Objectives: Investigate a potential impact of tumor-associated bacteria on neutrophil-mediated tumor progression and immune escape.

Materials and methods: We designed an in vitro system that mimics the cross-talk of tumor cells and neutrophils in the presence of bacterial stimulation. Stimulated tumor cells were tested in functional read-outs to determine migratory abilities and susceptibility to NK killing

Results: Bacterial stimulation enhanced the generation of an invasive and metastatic tumor cell phenotype via neutrophil activation. Metastatic tumor cells showed a strong mesenchymal phenotype, enhanced migratory abilities and resistance against NK cell cytotoxicity. Application of inhibitors led to the identification of distinct neutrophil granule proteins implicated in the induction of the pro-metastatic activity.

Conclusion: These data provide new insight into extrinsic mechanisms of HNC metastasis.

6

PD-L1 expression in the epithelial compartment at invasive tumour front is an independent prognostic biomarker in a cohort of oral squamous cell carcinoma Sudanese patients

N. Gaafar (Bergen/NO), T. Osman (Bergen/NO), M. Elsheikh (Khartoum/SD), I. Ahmed (Bergen/NO), H. Dongre (Bergen/NO), S. Fromreide (Bergen/NO), A. M. Suleiman (Khartoum/SD), A. C. Johannessen (Bergen/NO), E. S. Nginamau (Bergen/NO), D. E. Costea (Bergen/NO)

Background Invasive tumour front (ITF) has been proven as the most active part of OSCC lesions. However, few studies have assessed tumour immune microenvironment separately at ITF compared to tumour centre (TC). We aimed to evaluate patterns of immune cells infiltration at ITF in a cohort of OSCC patients from Sudan, and their association with infiltration patterns at their respective TC and with clinico-pathological parameters.

Methods This study was performed on a prospective cohort of 31 subjects attending Khartoum Dental Teaching Hospital (22 OSCC and 9 non-cancer participants) with a median follow-up of 48 months. Inflammatory infiltrate densities of CD4, CD8, FoxP3, CD20, CD66b, M1 (CD80/CD68), M2 (CD163/CD68) and PD-L1 positive cells at ITF and TC were assessed by immunohistochemistry followed by digital quantification at stromal and epithelial parts separately. Histopathological parameters such as worst pattern of invasion, differentiation, and tumour budding were also assessed. SPSS was used for statistical analyses.

Results Significantly more abundant inflammatory cell infiltrates of all subtypes investigated were observed at ITF in stromal compared to epithelial compartment, except PD-L1. The CD8 subtype was significantly higher in both stromal and epithelial compartments at ITF compared to TC, and CD8 stromal abundance at ITF associated with low TB. Intra-epithelial CD66b subtype was as well significantly higher in ITF than TC. Higher abundance of CD4 infiltrate and PD-L1 expression both in stromal and epithelial compartments at ITF associated with poor overall survival.

Conclusions Generally, more abundant inflammatory cell infiltrates were detected at ITF than TC in both stromal and intra-epithelial compartments, and abundance of certain subtypes of inflammatory cells correlated to overall survival only at ITF and not at TC. This indicates once again that ITF should be investigated when exploring different biomarkers for their prognostic value in OSCC.

7

Patient derived *ex vivo* tissue slice cultures demonstrate a profound DNA double-strand break repair defect in HPV-positive OPSCC

H. B. Zech (Hamburg/DE), C. S. Betz (Hamburg/DE), M. Kriegs (Hamburg/DE), S. Köcher (Hamburg/DE), J. Berger (Hamburg/DE), C. J. Busch (Greifswald/DE), A. Böttcher (Hamburg/DE), C. Petersen (Hamburg/DE), K. Rothkamm (Hamburg/DE), T. Rieckmann (Hamburg/DE)

Question: HPV-induced oropharyngeal cancer (OPC) are more sensitive towards radiation than HPV-negative. Underlying mechanism are discussed controversial. Theories are enhanced cellular radiosensitivity based on a defect in DNA double-strand break (DSB) repair or stronger immunogenicity. The mayor limitation of the first theory is the experimental restriction to a very limited number of HPV positive cell lines.

Methods: Fresh patient-derived OPSCC samples were cut in 400µm sections and cultured on cell culture inserts. Slice cultures were irradiated, in part combined with ATM inhibition, and fixed and frozen after 2 and 24 h. Residual DSBs were analyzed by quantification of 53BP1 foci in nuclei co-stained with the SCC marker p63 via immunofluorescence microscopy.

Results: Ex vivo OPSCC tumor slice cultures maintained stable oxygenation and proliferation characteristics for at least 3 days. p63-positive nuclei in HPV-positive OPSCC tissues (n=10) showed profoundly elevated numbers of residual radiation-induced DSBs as compared to those from HPV-negative OPSCC (n=10) (3Gy: on average 5.2 vs. 1.1 foci per nucleus; p=0.0003). Additional ATM inhibition resulted in a substantial increase in residual foci in all 4 HPV-negative samples tested but strikingly only in a single out of 9 HPV-positive samples.

Conclusions: In summary, our data provide robust, cell line-independent experimental evidence for an intrinsic DSB repair deficiency in HPV-positive OPSCC, strongly suggesting a meaningful contribution to the enhanced clinical radiosensitivity. The reduced effectiveness of ATM inhibition indicates a defect in the ATM-orchestrated DNA damage response. A low number of residual 53BP1 nuclear foci in the ex vivo assay may identify rare HPV-positive patients with effective DSB repair who should potentially be excluded from de-intensification approaches

8

Slug-related partial EMT is a transcriptomic prognosticator of head and neck cancer

O. Gires (Munich/DE), H. Schinke (Munich/DE), M. Pan (Munich/DE), P. Baumeister (Munich/DE), M. Canis (Munich/DE)

Question: Head and neck squamous cell carcinomas (HNSCC) are heterogeneous tumors with poor prognosis. Single-cell RNA sequencing (scRNAseq) revealed partial epithelial-to-mesenchymal transition (pEMT) as a central driver of cellular heterogeneity associated with nodal metastases and unfavorable clinical parameters in HNSCC (*1*). We aimed at transferring pEMT signatures from scRNAseq to bulk RNAseq data, which are more feasible for routine application.

Methods: scRNAseq signature-based pEMT quantification was developed through deconvolution of bulk RNAseq and micro-array data combined with single sample scoring of molecular phenotypes (Singscoring) for large HNSCC cohorts.

Results: Clinical pEMT-Singscores served as molecular classifier in multivariable Cox-proportional hazard models and prognosticated poor overall survival (OS) and reduced response to irradiation as independent parameters in large HNSCC cohorts (TCGA, MDACC, FHCRC). Differentially expressed genes (DEGs) confirmed enhanced cell motility and reduced oxidative phosphorylation and epithelial differentiation in pEMThigh patients. In patients and cell lines, EMT transcription factor (EMT-TF) SLUG correlated most strongly with pEMT-Singscores and promoted enhanced invasion and resistance to irradiation *in vitro*. SLUG protein levels in primary HNSCC predicted disease-free survival and its peripheral expression at the interphase to the tumor-microenvironment was significantly increased in relapsing patients.

Conclusions pEMT-Singscores represent a risk-predictor for HNSCC stratification regarding clinical outcome and therapy response that is partly controlled by SLUG (*2*).

1. S. V. Puram *et al.*, Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. *Cell* 171, 1611-1624 e1624 (2017).
2. H. Schinke *et al.*, SLUG-related partial epithelial-to-mesenchymal transition is a transcriptomic prognosticator of head and neck cancer survival. *Mol Oncol*, (2021).

9

^{68}Ga-FAPI PET/CT – a novel imaging approach to improve the specificity of cancer detection in patients with CUP or early oropharyngeal cancer

A. Scherzad (Würzburg/DE), S. Serfling (Würzburg/DE), Y. Zhi (Würzburg/DE), T. Gehrke (Würzburg/DE), M. Stöth (Würzburg/DE), F. Müller-Diesing (Aachen/DE), S. Hackenberg (Aachen/DE)

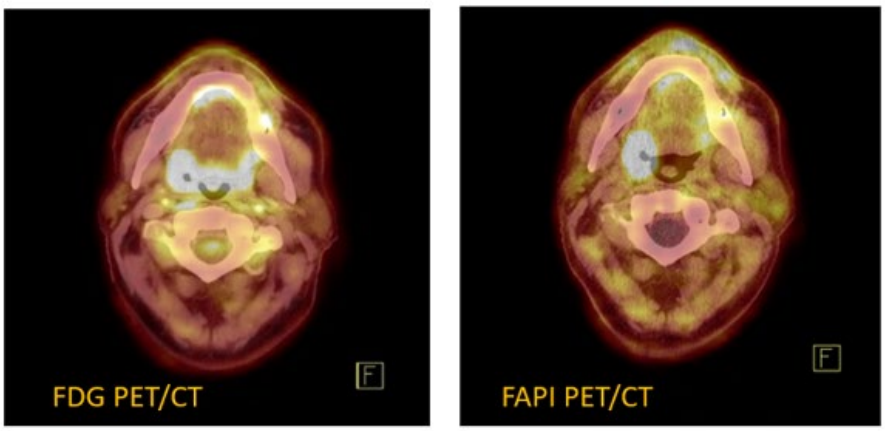
Objectives: 18F-FDG PET/CT represents a standard procedure for diagnostic imaging in CUP or HNSCC in general. 18F-FDG PET/CT shows a high sensitivity for cancer detection but there is a lack of differentiation between cancer and chronic inflammation, especially concerning lesions located in the Waldeyer"s tonsillar ring. Thus, small oropharyngeal cancer (OPC) lesion may be overseen. Fibroblast activation protein (FAP) is a cancer-specific protein, expressed by cancer-associated fibroblasts in HNSCC. In 68Ga-FAPI PET/CT, FAP is used as target of the PET radiotracer. In our study we compared sensitivity and especially specificity of this new approach to the established 18F-FDG PET/CT.

Methods: The study population consisted of eight patients with suspected OPC. All patients received preoperative PET/CT scans with 18F-FDG and 68Ga-FAPI. After surgical resection the diagnosis was confirmed histologically. Then, the uptake of the two different radiotracers in the tumor site and healthy contralateral tonsils were compared.

Results: Both tracers identified the primary tumors in all of the eight cases. The mean SUVmax of the primary tumors for 18F-FDG was 21.29 ± 7.97 and 16.06 ± 6.29 for 68Ga-FAPI ($p = 0.2$). The mean SUVmax of the healthy contralateral tonsils was 8.38 ± 2.45 for 18F-FDG and 3.55 ± 0.47 for 68Ga-FAPI ($p < 0.001$). The SUVmax ratio of 68Ga-FAPI was significantly different from 18F-FDG ($p = 0.03$). The mean tumor-to-background-ratio for the tracer 68Ga-FAPI was significantly higher compared to 18F-FDG (10.90 vs. 4.11).

Conclusion: It was shown that both 18F-FDG PET/CT and 68Ga-FAPI PET/CT are detecting OPC reliably. Moreover, the novel tracer shows a significantly higher specificity compared to the conventional 18F-FDG PET/CT. However, the 18F-FDG-PET/CT detection rate of cervical lymph node metastases smaller than 7 mm was higher than that of 68Ga-FAPI PET/CT. 68Ga-FAPI PET/CT is a notable instrument of cancer detection of patients with CUP or small OPC.

Figure



10

Linear regression and deep learning models for transcriptome-based HPV-status prediction in head and neck squamous cell carcinoma

E. Lombardo (Garching/DE, Munich/DE), J. Hess (Neuherberg/DE, Munich/DE), C. Kurz (Garching/DE, Munich/DE), M. Riboldi (Garching/DE), S. Marschner (Munich/DE, Neuherberg/DE), P. Baumeister (Neuherberg/DE, Munich/DE), K. Lauber (Munich/DE, Neuherberg/DE), U. Pflugradt (Munich/DE, Neuherberg/DE), A. Walch (Neuherberg/DE), M. Canis (Neuherberg/DE, Munich/DE), F. Klauschen (Neuherberg/DE, Munich/DE), H. Zitzelsberger (Neuherberg/DE, Munich/DE), C. Belka (Munich/DE, Neuherberg/DE), G. Landry (Garching/DE, Munich/DE), K. Unger (Neuherberg/DE)

HPV-status is a known prognostic factor for therapy outcome of head and neck squamous cell carcinomas. In this study we used transcriptome data of 348 patients with known HPV-status to build and compare the performance of regression-based and 2D Convolutional Neural Network (CNN) models for HPV prediction. For the CNN, transcriptome data was reorganized as 2D treemap images representing MSigDB Hallmark pathways. The treemaps were built three times in three different ways to assess the stability of the 2D-CNN. The features for the linear regression model were selected using Lasso on the training set. Applied on an unseen testing set comprising 25 % of the full patient dataset, the CNN achieved test ROC-AUCs/PR-AUCs of 0.95/0.87, 0.93/0.82 and 0.93/0.81 for the three variants of input treemaps respectively, while the regression model achieved a ROC-AUC/PR-AUC of 0.92/0.81. Therefore, we conclude that accurate predictions of HPV-status can be made with both models. However, the advantage over linear regression is that the deep learning model allows for functional interpretation through visualization of saliency maps computed using the Grad-CAM method.

11

Prognostic gene signature for squamous cell carcinoma with a higher risk for treatment failure and accelerated MEK-ERK pathway activity

B. Feng (Heidelberg/DE, Hangzhou/CN), E. Herpel (Heidelberg/DE), M. Plath (Heidelberg/DE), W. Weichert (Munich/DE), K. Freier (Homburg/DE), K. Zaoui (Heidelberg/DE), J. Hess (Heidelberg/DE)

Squamous cell carcinoma (SCC) is the most prevalent histological type of human cancer, including head and neck squamous cell carcinoma (HNSCC). However, reliable prognostic gene signatures for SCC and underlying genetic and/or epigenetic principles are still unclear. We identified 37 prognostic candidate genes by best cutoff computation based on survival in a pan-SCC cohort (n=1,334) of The Cancer Genome Atlas (TCGA), whose expression stratified not only the pan-SCC cohort but also independent HNSCC validation cohorts into three distinct prognostic subgroups. Most relevant prognostic genes were prioritized by a Least Absolute Shrinkage and Selection Operator Cox regression model and were used to identify subgroups with high or low risks for unfavorable survival. Integrative analysis of multi-omics data identified FN1, SEMA3A, CDH2, FBN1, COL5A1 and ADAM12 as key nodes in a regulatory network related to the prognostic phenotype. An in-silico drug screen predicted two MEK inhibitors (Trametinib and Selumetinib) as effective compounds for high-risk SCC based on the Cancer Cell Line Encyclopedia, which is supported by a higher p-MEK1/2 immunohistochemical staining of high-risk HNSCC. In conclusion, our data identified a molecular classifier for high-risk HNSCC as well as other SCC patients, who might benefit from treatment with MEK inhibitors.

12

Analysis of gene detection data of 115 cases of adenoid cystic carcinoma

X. CHEN (Beijing/CN)

Objective: This study analyze the mutation characteristics of 115 cases of Adenoid cystic carcinoma (ACC) gene and search for the driving gene.

Methods: 46 samples were tested using ACC specific panel (30 genes) and 69 samples were tested using large panel (559 genes /897 genes). In addition to, mutation signature analysis was performed on SNV mutations conforming to the filter criteria in 69 large panel sequencing samples using maftools software. Last, use MuSic2 software to analyze high-frequency mutation genes, to retain genes with Q-value<0.2 as high-frequency mutation genes, and use OncodriveCLUSTL and OncodriveFM to predict driver genes.

Results: Among 46 ACC customized panel samples, the detection rate of MYB-NFIB fusion was 36.96% (17/46), followed by TP53 15.22% (7/46), PTEN 8.7% (4/46) and NOTCH1 4.35% (2/46). Further, among 69 large panel test samples, the detection rate of MYB-NFIB fusion was 44.93% (31/69), followed by KMT5A 13.04% (9/69), TP53 13.04% (9/69), KMT2C 10.14% (7/69), NOTCH1 10.14% (7/69) and KDM6A 10.14% (7/69). In the next step, three mutations were identified: Signature 1 was present in all cancers and may be associated with spontaneous 5-methylcytosine deamination. Signature 6 is a DNA mismatch repair defect, indicating that ACC may have a DNA mismatch repair defect, and Signature 22 is aristolochic acid exposure. Finally, OncodriveCLUSTL and OncodriveFM predicted that GANS, AURKA, PTPN11, IDH2 and PTEN might be the driving genes.

Conclusions: MYB-NFIB is the most common mutation type of ACC. Importantly, these data suggest that ACC may be related to the characteristics of DNA mismatch repair and GANS, AURKA, PTPN11, IDH2 and PTEN may be the driving genes of ACC, but further research is needed.

13

Impact of radiation on function and apoptosis in peripheral CD8+ T lymphocytes in head and neck cancer patients

H. LIN (Shanghai/CN)

Introduction: Radiotherapy, which could lead local tumor destruction and abscopal effects in regional lesions, is a main treatment for head and neck squamous cell carcinoma (HNSCC). Nonetheless, peripheral CD8+ T lymphocytes are vulnerable to damage cause by radiation and could lead to immunosuppression.

Objective: The current study is designed to investigate the impact of radiation in peripheral CD8+ T lymphocytes in HNSCC patients.

Materials and methods: CD8+ T lymphocytes were collected from healthy donors (n=10), treatment-naïve HNSCC patients (n=10) and post-radiotherapy patients (n=5) and in vitro stimulated. Flow cytometry was used to analyze the expression of IFN-γ, TNF-α and perforin in CD8+ T lymphocytes. In vitro radiation (2Gy and 8Gy) was performed in peripheral CD8+ T lymphocytes from healthy donors (n=10) and treatment-naïve HNSCC patients (n=8). Flow cytometry and qRT-PCR were utilized to evaluate the proportion of apoptosis cells and the level of Bcl-2 family mRNA.

Results: After in vitro stimulation, the level of secreting cytokines between healthy donors and treatment-naïve patients showed no significant difference. The level of IFN-γ and TNF-α were significantly reduced in post-radiotherapy patients, while the level of perforin was similar between groups. The proportion of both early and late apoptosis cells was higher in CD8+ T lymphocytes from HNSCC patients after reception of in vitro radiation and the mRNA level of Bcl-2, Bcl-xL was decreased, while the level of BIM, BID, BAD and BAX was not significantly changed.

Conclusion: These findings demonstrated that radiotherapy could impair the secreting function of peripheral CD8+ T lymphocytes in HNSCC patients. Moreover, CD8+ T lymphocytes from HNSCC patients were more vulnerable to radiation-caused damage than healthy donors" counterparts.

14

CXCR4hiCD62Llo Aged Neutrophils in Laryngeal Squamous Cell Carcinoma Display an Immunosuppressive Phenotype

X. Zhu (Shanghai/CN)

Introduction: Tumor-associated neutrophils (TANs) has been shown to be associated with the prognosis of many tumors. Aged neutrophils represent a highly inflammatory subset of neutrophils purported to enhance the immunologic defense against invading pathogens, yet few studies have investigated their roles in laryngeal squamous cell carcinoma (LSCC).

Objectives: We aim to investigate the prognostic values of TANs in LSCC patients and explore the role of aged neutrophils in LSCC biology.

Materials and methods: An immunohistochemical analysis of LSCC microarrays was performed to assess the distribution and prognostic role of CD66b+ neutrophils. RNA-seq was performed to comprehensively analyze the genetic differences in TANs and peripheral blood neutrophils (PBNs). We then investigate the percentage of CXCR4hiCD62Llo aged neutrophils in peripheral blood from health donors and LSCC patients, as well as LSCC tissues and normal mucosa tissues from LSCC patients. The expression of immune checkpoint ligands on the cell surface of CXCR4hiCD62Llo aged neutrophils was further detected by flow cytometry.

Results: Generally, high infiltration of CD66b+ neutrophils in LSCC tissues showed a significant negative impact on overall survival ($p = 0.0018$). The RNA-seq data revealed a distinctly genetic difference between TANs and PBNs. The results of flow cytometry showed that compared with peripheral blood and normal mucosa tissue, the percentage of aged neutrophils was significantly up-regulated in the LSCC tissue ($p = 0.0032$). The expression of immune checkpoint ligands (e.g. PD-L1, CD155) was generally higher in TANs ($p = 0.028$). In addition, the subset of CXCR4hiCD62Llo aged neutrophils showed significantly more expressions of PD-L1 and CD155 than other subsets of neutrophils.

Conclusion: The increased infiltration of CXCR4hiCD62Llo aged neutrophils may exhibit robustly immunosuppressive phenotype and facilitate tumor growth.

15

Tumor-infiltrating antigen presenting cell composition in the human laryngeal squamous cell carcinoma

Y. Heng (Shanghai/CN)

Introduction: Cancer immunotherapy has revolutionized cancer treatment, and the immune landscape of the tumor microenvironment dominated by T lymphocytes shows great promise for the prediction of immunotherapy response and tumor prognoses. Although it is known that antigen-presenting cells (APCs) are key mediators of cellular cross-talk and are necessary for T-cell-mediated cancer immunity in the tumor microenvironment, the exact composition for tumor-infiltrating APC in laryngeal squamous cell carcinoma (LSCC) remains unclear.

Objectives: This study aims to reveal the tumor-infiltrating APCs composition in patients with LSCC.

Materials and methods: Double-labeling immunofluorescence (IF), immunohistochemical staining, and flow cytometry were used to characterize the immune infiltrate of APCs in LSCC tumors and adjacent normal tissues (ANTs).

Results: CD19+ B lymphocytes were the most common tumor-infiltrating APC cell type in LSCC tumors. Paraffin staining LSCC tumor tissues showed that CD19+ B cells were mainly distributed in the tumor stroma (TS) and less infiltrated in the tumor nest (TN). Although 86.1% of cases had CD19+ B cells infiltration in TS, only 20.3% had positive TN expression ($P<0.001$). Flow cytometry showed that tumor tissues had significantly higher expression of CD45+CD19+ B cells than ANTs ($P=0.015$). Among all subgroups of B cells, CD19-CD38hiCD27+ plasma cells and CD19+CD38hiIgG- germinal center B cells showed the most significant difference. Infiltration of iNOS+ M1 macrophage was observed in 16.3% cases and all of them were detected in TS, with none detected in TN and ANT iNOS+ M1 infiltration. However, TS infiltration of CD206+ M2 macrophage was observed in most cases. For DC subgroups, infiltration of PD-L2+ plasmacytoid dendritic cells was significantly higher in tumors than in ANTs.

Conclusion: Human LSCC tissues showed a remarkable APCs infiltration immune landscape and may play an important role in tumor progression of LSCC patients.

16

EGFR- and CD44v6-based CAR T-cell therapy for head and neck squamous cell carcinoma in Fanconi anemia

C. Haist (Essen/DE), A. Bister (Essen/DE), E. Schulte (Düsseldorf/DE), Z. Poschinski (Düsseldorf/DE), C. Wiek (Düsseldorf/DE), H. Hanenberg (Essen/DE), K. Scheckenbach (Düsseldorf/DE)

Fanconi anemia (FA) is a genetic disease associated with bone marrow failure and an increased incidence of squamous cell carcinomas, especially of the head and neck (HNSCC). Since therapeutic options are limited, we focused on the development of chimeric antigen receptors (CARs) expressing T-cells as an adoptive cellular therapeutic approach for HNSCCs in FA.

Study design: For two potential target antigens, CD44v6 and EGFR, the expression was measured on 33 primary HNSCC cell lines (FACS). CD44v6 is a CD44 splice variant that is formed in high concentrations in malignant cells and has been used as a target antigen for CAR T-cells in a phase I/II study against AML. EGFR is recognized by the approved monoclonal antibody cetuximab. 2nd generation CARs with high affinity were generated against both targets based on the antibodies BIWA8 (CD44v6) and cetuximab and primary human T-cells were transduced lentivirally. Finally, HNSCC cell lines with different levels of antigen expression were co-incubated with CAR T-cells for 16 hours and the lysis efficiency was determined (MTT assay).

Results: Both target antigens are highly expressed on most cell lines (FACS). Lysis efficiency of the CD44v6 CAR correlates well with the antigen density on the HNSCCs. In contrast, EGFR CAR T-cells also eliminate HNSCC cell lines with a low antigen density. Antigen is overexpressed using a cDNA and these isogenic cell triplets are used for the cytotoxicity assay. Cytotoxicity did not occur in the cells without expression of the target antigen and the overexpressing cells were eliminated very effectively. This proved the high specificity of both CAR constructs on allogeneic T-cells.

Conclusion: The high affinity EGFR and CD44v6 CAR T-cells are excellent effector cells against EGFR and CD44v6 expressing HNSCC cell lines. To further develop CAR T cells for FA patients, we will next examine the expression of both targets on FA HNSCC cell lines and then equip T-cells from FA patients with these CARs.

17

Neutrophils in tumor-draining lymph nodes control T-cell dependent anticancer immunity

E. Pylaeva (Essen/DE), G. Korschunow (Essen/DE), I. Spyra (Essen/DE), S. Bordbari (Essen/DE), E. Siakaeva (Essen/DE), I. Özel (Essen/DE), M. Domnich (Essen/DE), A. Squire (Essen/DE), A. Hasenberg (Essen/DE), K. Thangavelu (Essen/DE), T. Hussain (Essen/DE), M. Goetz (Essen/DE), K. S. Lang (Essen/DE), M. Gunzer (Essen/DE), W. Hansen (Essen/DE), J. Buer (Essen/DE), A. Bankfalvi (Essen/DE), S. Lang (Essen/DE), J. Jablonska (Essen/DE)

Tumor-draining lymph nodes (LNs) play a crucial role during cancer spread and in initiation of anti-cancer adaptive immunity. Here, we examined the role of LN microenvironment in tumor-supportive polarization of infiltrating neutrophils in head and neck cancer (HNC).

In total, 121 patients with HNC were enrolled; blood, tumor, LNs were obtained and grouped according to the disease stage, early, non-metastatic (N0) and late, metastatic (N1-3); metastasis-free and metastatic LNs in the latter group were examined separately. Cytokines and chemokines expression profile in LN-conditioned medium was assessed, the amount and spatial distribution of neutrophils in LNs were evaluated in histological images, activatory status of LN-infiltrating neutrophils and T-cells was estimated with flow cytometry and correlated with clinical data and survival.

Neutrophils in LNs shape anti-tumor responses in stage-dependent manner. In N0 patients, neutrophils develop antigen-presenting phenotype ($HLA\text{-}DR^{+}CD80^{+}CD86^{+}ICAM1^{+}PD\text{-}L1^{-}$) and stimulate T-cells ($CD27^{+}Ki67^{high}PD\text{-}1^{-}$). The presence of LN metastases triggers the development of $PD\text{-}L1^{+}$ immunosuppressive neutrophils, which repress T-cell responses. The accumulation of neutrophils in T-rich zones of LNs in N0 patients constitutes a positive predictor for 5-years survival, while the increased numbers of neutrophils in LNs of N1-3 patients serve as predictor for poor prognosis in HNC.

These results suggest a dual-edged role of neutrophils as essential regulators of anti-cancer immune responses in LNs and argue for approaches fostering immunostimulatory activity of these cells during cancer therapy.

18

Head and neck cancer stem cell vaccine significantly reduces local tumor relapse and prolongs survival in the adjuvant setting

Q. Li (Ann Arbor, MI/US)

Although surgical resection has been a standard treatment for head and neck cancer, therapeutic efficacy is limited by both local and distant recurrence. Effectively preventing local tumor recurrence remains a significant challenge. The existence of micro metastasis at the time of tumor resection represents an even greater therapeutic challenge. Targeting cancer stem cells (CSCs) may thus increase the therapeutic efficacy of current head and neck cancer treatment. We previously described a strategy to target head and neck squamous cell cancer (HNSCC) ALDHhigh CSCs using CSC-dendritic cell (DC) vaccination. However, the efficacy of CSC targeted therapeutics may be greatest when they are deployed in the adjuvant setting. In this study, established s.c. SCC7 (murine HNSCC) tumors were surgically removed from mice followed by treatment using ALDHhigh SCC7 CSC-DC vaccine, which significantly reduced local tumor relapse and prolonged animal survival. This effect was significantly augmented by simultaneous administration of anti-PD-L1 mAb. These results were confirmed in mouse D5 melanoma model. CCR10 and its ligands were down-regulated on ALDHhigh CSCs and in lung tissues respectively in animals subjected to ALDHhigh CSC–DC vaccination. Down-regulation of CCR10 by siRNA significantly blocked tumor cell migration *in vitro* and metastasis *in vivo.* T cells harvested from ALDHhigh CSC–DC vaccinated animals selectively killed the ALDHhigh CSCs. There was also evidence of humoral immunological targeting of CSCs. As a result, CSC-DC vaccination significantly decreased the percentage of ALDHhigh cells in residual tumors. These data indicate that, when used in an adjuvant setting, ALDHhigh CSC–DC vaccines effectively inhibit local tumor recurrence and prolong animal survival, compared with traditional DC vaccines and that simultaneous PD-L1 blockade can significantly enhance this effect.

19 - withdrawn

20

Transcriptional subtypes in matched primary and recurrent head and neck squamous cell carcinomas

P. Weber (Neuherberg/DE), A. Künstner (Lübeck/DE), J. Hess (Neuherberg/DE, Munich/DE), K. Unger (Neuherberg/DE, Munich/DE), S. Marschner (Neuherberg/DE, Munich/DE), C. Idel (Lübeck/DE), J. Ribbat-Idel (Lübeck/DE), P. Baumeister (Neuherberg/DE, Munich/DE), O. Gires (Neuherberg/DE, Munich/DE), M. Canis (Neuherberg/DE, Munich/DE), K. Lauber (Neuherberg/DE, Munich/DE), B. Wollenberg (Lübeck/DE, Munich/DE), H. Busch (Lübeck/DE), C. Belka (Neuherberg/DE, Munich/DE), H. Zitzelsberger (Neuherberg/DE, Munich/DE)

Introduction and objectives: Patients suffering from advanced head and neck squamous cell carcinomas (HNSCC) are at high risk for developing local and loco-regional recurrences after radiochemotherapy. Hitherto, current treatment of recurrent HNSCC ignores molecular characteristics of resistant tumors.

Materials and methods: In this study, we investigated matched pairs (n=38) of primary/recurrent tumors by exome and whole RNA sequencing and characterized the mutational landscape and the predominant transcriptional subtypes by using an established subtype classification (classical/CL, basal/BA, inflamed-mesenchymal/IMS subtypes).

Results: The mutational and genomic copy number patterns of primary and recurrent tumors were mostly discordant and showed only a low relationship between pairs of primary and relapsed tumors. CL and IMS subtypes were more common in HNSCC with low recurrence rates, while the BA subtype was prevalent and stable in recurrent tumors. 15 out of the 34 tumor pairs exhibited a change in transcriptional subtype between primary and recurrent tumor. Eight of these subtype switchers changed the molecular subtype from IMS to either CL or BA. Gene set enrichment analysis showed an upregulation of gene signatures for hypoxia, p-EMT and radiation resistance in recurrent compared to primary tumors. At the same time a tumor inflammation signature was downregulated in recurrences compared to index tumors.

Conclusion: Our study showed a high degree of heterogeneity between index tumors and recurrences and a therapy-induced occurrence of a transcriptional subtype with unfavorable outcome after therapy. Therefore, therapy decisions should consider genetic and transcriptomic characteristics of recurrent tumors to facilitate tailored treatment strategies.

21

Integration of p16/HPV DNA status with a 24-miRNA-defined molecular phenotype improves clinically relevant stratification of head and neck cancer patients

J. Hess (Neuherberg/DE, Munich/DE), K. Unger (Neuherberg/DE, Munich/DE), C. Maihöfer (Neuherberg/DE, Munich/DE), L. Schüttrumpf (Neuherberg/DE, Munich/DE), P. Weber (Neuherberg/DE), S. Marschner (Neuherberg/DE, Munich/DE), P. Baumeister (Neuherberg/DE, Munich/DE), A. Walch (Munich/DE), C. Woischke (Munich/DE), T. Kirchner (Munich/DE, Heidelberg/DE), M. Werner (Freiburg/DE, Heidelberg/DE), K. Sörensen (Freiburg/DE, Heidelberg/DE), M. Baumann (Heidelberg/DE, Dresden/DE), I. Tinhofer-Keilholz (Berlin/DE, Heidelberg/DE), S. E. Combs (Heidelberg/DE, Munich/DE), J. Debus (Heidelberg/DE), H. Schäfer (Heidelberg/DE, Freiburg/DE), M. Krause (Heidelberg/DE, Dresden/DE), A. Linge (Heidelberg/DE, Dresden/DE), J. von der Grün (Frankfurt a. M./DE, Heidelberg/DE), M. Stuschke (Essen/DE, Heidelberg/DE), D. Zips (Tübingen/DE, Heidelberg/DE), M. Canis (Neuherberg/DE, Munich/DE), K. Lauber (Neuherberg/DE, Munich/DE), U. Ganswindt (Neuherberg/DE, Munich/DE, Innsbruck/AT), M. Henke (Heidelberg/DE, Freiburg/DE), H. Zitzelsberger (Neuherberg/DE, Munich/DE), C. Belka (Neuherberg/DE, Munich/DE, Heidelberg/DE)

Introduction and objectives: Human papillomavirus (HPV) associated head and neck squamous cell carcinomas (HNSCC) generally have a more favourable prognosis. We hypothesized that HPV-associated HNSCC may be identified based on a miRNA signature according to their specific molecular pathogenesis and are characterized by a unique transcriptome compared to HPV-negative HNSCC.

Materials and methods: miRNA expression was analysed by microarrays in p16/HPV DNA characterized HNSCC of the multicentre DKTK-ROG (n=128) and the single-centre LMU-KKG (n=101) cohorts of patients treated by surgery and adjuvant radio(chemo)therapy. A linear model predicting HPV-status was built in DKTK-ROG using lasso-regression and tested in LMU-KKG. RNAseq data was generated for LMU-KKG tumours with the 15 highest and lowest HPV prediction scores.

Results: A 24-miRNA signature predicted HPV-status with 94.53% accuracy (AUC: 0.99) in DKTK-ROG and 86.14% (AUC: 0.86) in LMU-KKG. The prognostic values of 24-miRNA- and p16/HPV DNA status were comparable. Sub-stratification of patients by combining p16/HPV DNA and 24-miRNA signature status allowed the identification of an HPV-associated HNSCC patient subgroup with impaired overall survival. HPV-positive tumours showed downregulated MAPK, Estrogen, EGFR, TGFbeta and WNT signalling activity. miRNA-mRNA integration revealed HPV-specific regulation of signalling pathways, including PD−L1 expression/PD−1 checkpoint pathway in cancer in HPV-associated HNSCC.

Conclusion: Integration of clinically established p16/HPV DNA status with a 24-miRNA signature improves clinically relevant risk stratification. Functional assignment of signature miRNAs suggests an important post-transcriptional integrative role in key deregulated pathways in HNSCC. Future clinical decision-making on treatment de-escalation in HPV-positive HNSCC might consider 24-miRNA signature classification combined with p16/HPV DNA status.

22

Exosome-mediated radiation-induced bystander effect in human head and neck cancer cells in vitro

M. Smolarz (Gliwice/PL), A. Abramowicz (Gliwice/PL), M. Pietrowska (Gliwice/PL), P. Widlak (Gliwice/PL)

Exosomes are key mediators of cell-to-cell communication involved in different aspects of the response to ionizing radiation. It has been hypothesized that exosomes released by irradiated cells propagate the radiation-induced bystander effect (RIBE) to naïve recipient cells. Here we aimed to analyze the protein and miRNA profile of exosomes released by cells irradiated *in vitro*. Cells derived from human head and neck cancer (FaDu and UM-SCC6) were irradiated with a single 2 Gy dose then exosomes were purified from culture media at 24 h post-irradiation using size-exclusion chromatography. Protein and miRNA content of exosomes was analyzed using the LC-MS and the RNA-seq approach, respectively; exosomes released by irradiated cells and control not irradiated cells were analyzed in parallel. Proteins upregulated in exosomes released by irradiated cells were involved in the response to radiation, the metabolism of radical oxygen species, DNA repair, chromatin packaging, and protein folding. Radiation-affected miRNA species present in exosomes were linked to genes involved in the DNA damage and cytokine-mediated response. Further, the effect of exosome uptake on the phenotype of the naïve recipient FaDu was addressed. Exosomes released by irradiated cells induced the RIBE in recipient cells, which was reflected by the accumulation of the γH2A.X foci (the indicator of activation of the signaling induced by DNA breaks) after 1 to 3 hours of incubation. Moreover, foci of phosphorylated 53BP1 protein, a key DNA damage checkpoint signal, were induced in recipient cells. Furthermore, the accumulation of DNA:RNA hybrids were observed, which suggested that exosome-mediated signals induced replication stress in recipient cells. Hence, the molecular content of exosomes released by irradiated cells fitted to their hypothetical role in the mediation of RIBE. Moreover, naïve cells that received exosomes released by irradiated cells activated signaling characteristics for DNA breaks.

23

Early senescence and production of senescence-associated cytokines are major determinants of radioresistance in head-and-neck squamous cell carcinoma

U. Schoetz (Munich/DE), D. Klein (Essen/DE), J. Hess (Oberschleißheim/DE), S. Shnayien (Munich/DE), S. Spoerl (Munich/DE), M. Orth (Munich/DE), S. Mutlu (Munich/DE), R. Hennel (Munich/DE), A. Sieber (Berlin/DE), U. Ganswindt (Munich/DE), B. Luka (Freiburg/DE), A. Thomsen (Freiburg/DE), V. Jendrossek (Essen/DE), K. Unger (Oberschleißheim/DE), H. Zitzelsberger (Oberschleißheim/DE), N. Blüthgen (Berlin/DE), C. Belka (Munich/DE), S. Unkel (Göttingen/DE), B. Klinger (Berlin/DE), K. Lauber (Munich/DE)

Introduction: Resistance against radio(chemo)therapy is a major determinant of oncological treatment failure and a perpetual clinical challenge. The underlying mechanisms are complex and demand for comprehensive examination.

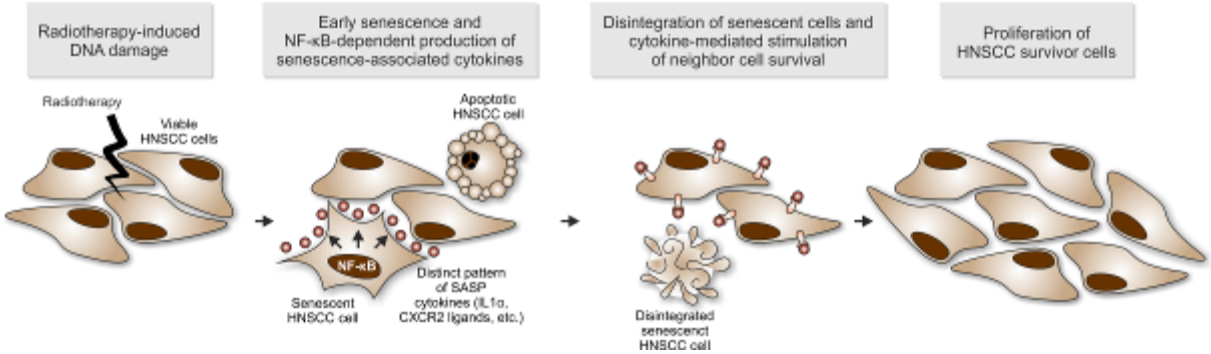
Objectives: The present study was designed to systematically characterize radioresistance in model systems of head and neck squamous cell carcinoma (HNSCC) with the superordinate aim to extract potential vulnerabilities.

Materials and methods: A panel of HNSCC cell lines and xenotransplants derived thereof was characterized with regard to clonogenic survival upon radiotherapy, transcriptomic profiling, and different cell fate decisions. Integration of the respective datasets was employed to identify associations.

Results: Integration of clonogenic survival data with molecular and functional data on DNA damage repair and different cell fate decision revealed a positive correlation between radioresistance and early induction of HNSCC cell senescence. This was accompanied by the NF-κB-dependent production of distinct senescence-associated cytokines, particularly ligands of the CXCR2 chemokine receptor. Time-lapse microscopy and medium transfer experiments disclosed the non-cell autonomous nature of these mechanisms, and pharmacological interference with senescence-associated cytokine production by the NF-κB inhibitor metformin significantly improved radiotherapeutic performance *in vitro* and *in vivo*. Retrospective analyses of TCGA HNSCC data and an in-house HNSCC cohort revealed that elevated expression of CXCR2 and/or its ligands is associated with impaired treatment outcome.

Conclusion: Our study identifies radiation-induced tumor cell senescence and the NF-κB-dependent production of distinct senescence-associated cytokines as drivers of radioresistance in HNSCC whose therapeutic targeting in multi-modality treatment approaches should be further examined and may be of particular interest for HNSCCs with elevated expression of the CXCR2/ligand axis.

Figure



P24 - withdrawn

P25

cfHPV16-DNA in liquid biopsy – a promising biomarker for patients with HPV-related oropharyngeal squamous cell carcinoma

N. Würdemann (Cologne/DE), C. Alidousty (Cologne/DE), O. G. Siefer (Cologne/DE), R. Jain (Cologne/DE), C. Huebbers (Cologne/DE), S. Silling (Cologne/DE), D. Schafigh (Cologne/DE), R. P. Bacher (Cologne/DE), H. F. S. Reder (Gießen/DE), R. Büttner (Cologne/DE), M. Dietlein (Cologne/DE), U. Wieland (Cologne/DE), A. Drzezga (Cologne/DE), M. Schlamann (Cologne/DE), A. Quaas (Cologne/DE), C. Wittekindt (Dortmund/DE), E. J. M. Speel (Maastricht/AN), S. Wagner (Gießen/DE), A. M. Schultheis (Cologne/DE), J. P. Klußmann (Cologne/DE)

Introduction: Incidences of high-risk Human Papillomavirus (HPV) oropharyngeal squamous cell carcinoma (OPSCC) are increasing worldwide. Although HPV-related OPSCC patients display a significantly better 5-year overall survival compared to HPV-negative OPSCC, up to 25% develop locoregional recurrence or distant metastases. Biomarkers to monitor the course of disease are lacking to this point.

Objectives: The aim of this study was to correlate copy number of cell-free (cf)HPV16 DNA in plasma with treatment response and disease progression of OPSCC patients to investigate its function as biomarker.

Material and methods: In a multicenter study, the concentration of cfHPV16-E6/E7 DNA in plasma was measured by quantitative (q)PCR / droplet digital (dd)PCR at diagnosis and during follow-up in patients with OPSCC (test-cohort n = 37; validation-cohort n = 53). Copy number of cfHPV16-E6/E7 DNA in plasma was subsequently correlated with the course of disease as well as imaging and clinicopathological parameters.

Results: Receiver Operating Characteristic (ROC)-Analysis revealed an outstanding discrimination of cfHPV16-DNA (E7) in ddPCR (AUC = 0.9467) and qPCR (AUC = 0.9500) in comparison to HPV-status of the primary in the test-cohort. Elevated cfHPV16-DNA E7 copy number detected by ddPCR correlated with higher T-stage ($p \leq 0.050$). In our validation-cohort, ROC-Analysis revealed an outstanding discrimination only in E6-ddPCR (AUC = 0.9256) and correlation of median cfHPV16-DNA copy number with N-stage was detected via qPCR method. Furthermore, cfHPV16-DNA copy number correlated with metabolic tumor volume in 18F-FDG-PET-CT ($r = 0.5982$; $R2 = 0.3578$; $p = 0.0012$). In both cohorts a significant reduction in cfHPV16 E6/E7 DNA levels was detected post-therapeutically ($p \leq 0.001$), whereas an increase in copy number correlated with development of recurrence.

Conclusion: cfHPV16-DNA in plasma of patients with HPV-related OPSCC presents a promising biomarker for disease monitoring.

P26

Matrix metalloproteinases enriched in tumor-derived small extracellular vesicles promote progression of head and neck squamous cell carcinoma by reprogramming of macrophages

T. Kärmer (Regensburg/DE), K. Franke (Regensburg/DE), A. Gluszko (Warsaw/PL), J. Eichberger (Regensburg/DE), D. Schulz (Regensburg/DE), D. Froschhammer (Regensburg/DE), M. Szczepański (Warsaw/PL), T. Reichert (Regensburg/DE), R. Bauer (Regensburg/DE), N. Ludwig (Regensburg/DE)

Introduction: Tumor-derived small extracellular vesicles (TEX) are secreted by head and neck squamous cell carcinoma (HNSCC) cells and carry a complex cargo composition which reflects the status of the parent cells. Some cargo components of TEX were shown to contribute to cell-to-cell communication and ultimately promote disease progression.

Objectives: The aim of this study was to detect matrix metalloproteinases (MMPs) in TEX and characterize their functional effects on primary human macrophages.

Materials and methods: TEX were isolated from supernatants of UMSCC9 and UMSCC47 HNSCC cells by size exclusion chromatography and characterized by nanoparticle tracking analysis, electron microscopy, and immunoblotting. MMP-2 and MMP-9 were detected in TEX by immunoblotting and enzymatic activity was determined by zymography. Primary human macrophages were generated from monocytes of healthy donors by treatment with granulocyte-macrophage colony stimulating factor (GM-CSF). Macrophages were co-incubated with TEX and the phenotype and secretome was assessed.

Results: Isolated TEX were morphologically and functionally intact and ranged in size from 30-150 nm. Immunoblotting revealed the presence of TEX markers TSG101, HSP70, and CD9 and the absence of the negative marker Calnexin. TEX carried enzymatically-active MMP-2 and MMP-9, which promoted macrophage polarization to a mixed M1/M2 phenotype with a trend towards M2. TEX were internalized by macrophages and stimulated the release of pro-angiogenic factors which promoted proliferation ($p < 0.05$) and migration ($p < 0.01$) of endothelial cells.

Conclusion: The results indicate that MMPs are enriched in TEX and are involved in functional reprogramming of macrophages. Thus, TEX emerge as an important source for MMPs in the tumor microenvironment and as a potential therapeutic target in HNSCC.

P27
POFUT1 acts as an oncogene in human laryngeal squamous cell carcinoma

O. F. Karatas (Erzurum/TR), N. Barlak (Erzurum/TR), N. Demiroglu (Erzurum/TR), A. Tatar (Erzurum/TR), B. Gundogdu (Erzurum/TR)

Introduction: Although there have been significant improvements in the detection and diagnosis of LSCC in the last decades, it is still one of the considerable causes of cancer deaths and an urgent need for development of novel therapeutic approaches against advanced LCa cases still remains. POFUT1 gene resides within 20q11.21 chromosomal region that is a recurrent gain of function abnormality in human embryonic stem cells (hESCs). Interestingly, 20q11.21 amplification in hESCs resulted in acquisition of a gene-expression signature enriched for cancer-associated genes and POFUT1 been shown to be upregulated in several cancers.

Objectives: Here, in this study, we aimed at investigating the oncogenic potential of POFUT1 in LCa.

Materials and methods: We examined the expression of POFUT1 in tissue samples using *in silico* tools, quantitative real-time polymerase chain reaction (qRT-PCR), and Western Blot. We tested its *in vitro* oncogenic potential in LCa cells using cell viability, migration and invasion assays. We analyzed the regulatory roles of miR-1825 on the stemness features of LCa cells using colony formation, sphere formation and single cell colony assays and examined the expression of genes related to stemness and epithelial mesenchymal transition (EMT) in cells transfected with either miR-1825 mimic/inhibitor using qRT-PCR.

Results: We showed that POFUT1 is significantly upregulated in LCa tumor tissue samples compared with the corresponding controls. *In vitro* assays revealed that POFUT1 promoted proliferative, migratory, and invasive potential in LCa cells. We also showed that miR-1825 significantly enhanced stemness features in LCa cells and also induced the expressions of genes related to both stemness and EMT.

Conclusion: We propose that POFUT1 carries crucial roles in laryngeal tumorigenesis and might serve as an important target for therapy.

This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number 118Z767).

P28

Tumor expression of a gene set that correlates with extracellular vesicle production shapes the immune landscape in head and neck squamous cell carcinoma

I. Kallinger (Regensburg/DE), G. Spanier (Regensburg/DE), S. Spoerl (Regensburg/DE), T. Reichert (Regensburg/DE), J. Meier (Regensburg/DE), N. Ludwig (Regensburg/DE)

Introduction: Head and neck squamous cell carcinomas (HNSCCs) are characterized by an immunosuppressive environment and evade immune responses through multiple resistance mechanisms. Tumor-derived small extracellular vesicles (EVs) were recently described to be potentially engaged in negative regulation of antitumor immune responses due to their suppression of immune cell subsets.

Objectives: The goal of this study was to evaluate the clinical relevance of HNSCC expression levels of a gene set that is associated with EV production and correlate gene expression levels with infiltration abundances of immune cell subsets.

Materials and methods: Gene expression levels of a recently published gene set (Fathi *et al.* Cancers 2021) were determined based on the Cancer Genome Atlas (TCGA) Head and Neck Cancer data base. In total, 522 cases of primary HNSCC were included in this study and compared to 44 normal controls. 98 cases of HPV(+) HNSCC and 422 cases of HPV(−) HNSCC were used for further analysis. Potential associations between gene expression levels, immune cell infiltration, and patient overall survival (OS) were analyzed by TIMER2.0 and the UCSC Xena browser.

Results: In comparison to normal control tissue, 19 of the 41 EV-associated genes were upregulated in HNSCC tissue. Expression levels of the majority of genes in HPV(-) patients were linked to poor OS, whereas expression levels correlated with improved OS in HPV(+) patients. Expression levels of the EV-associated gene set correlated with elevated infiltration abundances of CD4(+) T cells, macrophages, neutrophils, and dendritic cells into tumor tissues (p<0.01). Interestingly, elevated gene expression was also associated with an exclusion of B cells and CD8(+) T cells in tumor tissue (p<0.01).

Conclusion: Enhanced tumor expression of a gene set that correlates with EV production is associated with a specific immune cell composition characterized by reduced tumor-infiltrating effector lymphocytes.

P29

Development of an antibody panel for imaging mass cytometry to investigate cancer-associated fibroblast heterogeneity in archival tissues

S. Tornaas (Bergen/NO), S. Fromreide (Bergen/NO), H. Y. Smeland (Bergen/NO), H. J. Aarstad (Bergen/NO), O. K. Vintermyr (Bergen/NO), L. A. Akslen (Bergen/NO), H. Dongre (Bergen/NO), D. E. Costea (Bergen/NO)

Introduction: It is now generally accepted that cancer-associated fibroblasts (CAFs) contribute several factors for tumor development and progression. Studies aimed at depleting CAFs have had poor outcomes. This might be attributed to various phenotypes and functions of CAFs. We have previously described two functionally distinct CAF phenotypes in head and neck carcinoma, but not a full phenotype of subpopulations of CAFs. The aim for this study was to develop a novel panel of antibody (AB) for imaging mass cytometry (IMC) to distinguish different CAF phenotypes on formalin-fixed paraffin-embedded (FFPE) archival tissues.

Methods: A comprehensive panel of ABs were chosen based on known CAF markers from literature and the ABs were conjugated to rare earth metals using the protocol provided by the supplier (Fluidigm, South San Francisco, CA, USA). FFPE tissue samples from different organs/lesions known to express certain markers were used for the generation of control tissue microarrays (cTMAs) for verification of the conjugated ABs. Immunohistochemistry (IHC) with metal-conjugated Abs was used as the "golden standard" for optimization of IMC.

Results: The performance of conjugated ABs was verified by performing IHC and IMC. The results showed that all in-house and pre-conjugated antibodies worked satisfactorily by both methods, but standardization of the concentration of ABs and incubation time was necessary for obtaining similar staining patterns by IHC and IMC.

Conclusion: This study shows that it is feasible to use an extensive panel of ABs for the characterization of CAF phenotypes using metal tags.

P30

Potential role of small extracellular vesicles in reprogramming neutrophil functions in head and neck squamous cell carcinoma

K. Franke (Regensburg/DE), D. Rubenich (Porto Alegre/BR), T. Kärmer (Regensburg/DE), A. Gluszko (Warsaw/PL), D. Schulz (Regensburg/DE), M. Szczepański (Warsaw/PL), M. Fiedler (Regensburg/DE), G. Spanier (Regensburg/DE), S. Spoerl (Regensburg/DE), E. Braganhol (Porto Alegre/BR), T. Reichert (Regensburg/DE), R. Bauer (Regensburg/DE), N. Ludwig (Regensburg/DE)

Introduction: Tumor-derived small extracellular vesicles (TEX) have recently been identified as important mediators of intercellular communication in head and neck squamous cell carcinoma (HNSCC). The interaction of TEX with recipient cells in the tumor microenvironment (TME) and distant sites was shown to result in promotion of disease progression. One important cellular component of the TME are neutrophils which are phenotypically and functionally reprogrammed by the tumor resulting in poor patient survival.

Objectives: The goal of this study was to characterize the crosstalk between HNSCC cells and neutrophils via TEX.

Materials and methods: TEX were isolated from supernatants of UMSCC47 HNSCC cells by size exclusion chromatography and characterized by nanoparticle tracking analysis, electron microscopy, immunoblotting, and functional studies. Primary human neutrophils were isolated from healthy donors and analyzed in functional studies in the presence or absence of TEX. Conditioned medium (CM) was harvested from neutrophils after co-incubation with TEX and was used for proliferation and migration studies with UMSCC47 cells.

Results: Isolated TEX were morphologically intact, non-aggregated, and functionally competent. TEX ranged in size from 30–150 nm and carried positive TEX markers TSG101, HSP70, and CD9, whereas the negative marker Calnexin was absent. TEX stimulated neutrophil survival in a concentration dependent manner ($p < 0.0001$). CM of neutrophils reprogrammed by TEX had no significant effects on tumor cell proliferation, but stimulated tumor cell migration in wound healing assays ($p < 0.05$).

Conclusion: The results indicate that TEX contribute to the generation and maintenance of tumor-associated neutrophils ultimately stimulating disease progression in HNSCC. Future efforts should focus on further characterizing the proteomic, genomic, and functional alterations of neutrophils which are potentially induced by TEX.

P31

An estrogen receptor 1-related risk model identifies HNSCC patients with a favorable immune phenotype and characteristic molecular features

F. Almouhanna (Heidelberg/DE), K. Khorani (Heidelberg/DE), P. K.Plinkert (Heidelberg/DE), J. Hess (Heidelberg/DE)

Introduction: Head and neck squamous cell carcinomas (HNSCC) are among the deadliest cancers, and multiple risk factors, molecular traits and clinical features affect the response to treatment and thus the overall survival. Studies have emerged describing a positive correlation between the expression of estrogen receptor 1 (ESR1), HPV16 status and favorable overall survival in HNSCC. However, ESR1 expression does not necessarily correlate with its cellular function and underlying molecular principles remain elusive.

Objectives: This study aims to establish an ESR1-related gene signature to stratify HNSCC patients at higher risk for treatment failure, who might benefit from new treatment options.

Materials and methods: RNA-seq data from TCGA-HNSC were used as a training cohort to identify an ESR1-related gene set. A risk model for survival was established by LASSO-Cox regression and confirmed in independent HNSCC cohorts and other tumors of TCGA. Differences in the mutational landscape, gene regulatory networks and signaling pathways among risk groups were analyzed by bioinformatics approaches.

Results: Our analysis revealed 139 ESR1-related genes in TCGA-HNSC and a risk model based on a 25-gene set with highly significant prognostic value, which was confirmed in independent HNSCC cohorts and other tumors of TGCA. By performing a comprehensive comparison between the low and high-risk groups, somatic mutations (NSD1), copy number variation (genome instability), and the activation status of immune-related pathways were significantly different. Interestingly, multiple immune-related pathways were enriched in the low-risk group, representing high T cell activity independent of the HPV16 status.

Conclusion: Our data provide compelling evidence on the association between an ESR1-related pathway activity and a more favorable immune phenotype, indicating new strategies for more effective and personalized therapies of HNSCC patients

P32

Liquid biopsy for minimal residual disease detection in head and neck squamous cell carcinoma (LIONESS) – personalised circulating cell-free tumour DNA analysis for detection of minimal residual disease and recurrence in patients with HNSCC

S. Flach (Munich/DE), K. Howarth (Cambridge/GB), S. Hackinger (Cambridge/GB), C. Pipinikas (Cambridge/GB), P. Ellis (Cambridge/GB), K. McLay (Cambridge/GB), G. Marsico (Cambridge/GB), C. Walz (Munich/DE), C. Reichel (Munich/DE), O. Gires (Munich/DE), M. Canis (Munich/DE), P. Baumeister (Munich/DE)

Introduction Circulating cell-free tumour DNA (ctDNA) is a recently identified biomarker which remains largely uncharacterised in the context of surgical treatment of patients with head and neck squamous cell carcinoma (HNSCC). The aims of this study were firstly to determine whether post-operative ctDNA detection can act as a biomarker for surgical tumour clearance. Secondly, to evaluate the potential of personalised ctDNA analysis for early molecular-level detection of relapse prior to clinically confirmed recurrence.

Methods We conducted LIONESS, a single-centre prospective cohort study to investigate ctDNA in patients with p16-negative HNSCC who received primary surgical treatment with curative intent. Whole exome sequencing was performed on FFPE tissue obtained from 25 unique tumours. We utilised RaDaR™, a highly sensitive personalised assay using deep sequencing of up to 48 tumor-specific variants. The RaDaR™ assay demonstrated 95% sensitivity at 0.001% median variant allele frequency in analytical validation studies and was used to analyse serial pre- and post-operative plasma samples for evidence of minimal residual disease and recurrence.

Results In a subset of 21 patients analysed, personalised panels were designed with between 20 and 52 somatic variants (median 48). Preliminary data shows 100% ctDNA detection in baseline samples taken prior to surgery. 131 longitudinal samples were assessed for evidence of ctDNA. In post-surgery samples, ctDNA could be detected at levels as low as 0.0005% AF. In all cases with clinical recurrence to date (5/5), ctDNA was detected prior to progression, with lead times ranging from 108 to 298 days.

Conclusion This study illustrates the potential of ctDNA as a biomarker for monitoring of minimal residual disease as well as recurrence in patients with HNSCC and demonstrates the feasibility of personalised ctDNA assays for detection of disease post-treatment and with consequences for further therapy planning.

P33

Cell-intrinsic function and regulation of the immune checkpoint regulator PD-L1 in HNSCC

D. Schulz (Regensburg/DE), J. Eichberger (Regensburg/DE), M. Fiedler (Regensburg/DE), I. Stancev (Regensburg/DE), M. Streller (Regensburg/DE), M. Wetzel (Regensburg/DE), K. Pscheidl (Regensburg/DE), L. Feulner (Regensburg/DE), G. Piendl (Regensburg/DE), G. Brockhoff (Regensburg/DE), M. Hautmann (Regensburg/DE), T. Reichert (Regensburg/DE), R. Bauer (Regensburg/DE), T. Ettl (Regensburg/DE)

Introduction: Immunotherapy by blockade of the PD-1/PD-L1 checkpoint demonstrated amazing tumor response in advanced cancer patients including HNSCC. The majority of patients show little improvement or hyperprogression. Irradiation is a synergistic treatment modality to immunotherapy. Recently, a growing amount of data indicated that PD-L1 also plays an intrinsic cellular role by regulating different functions like cell proliferation migration or invasion.

Objectives: The aim of this study was to further elucidate the cell-intrinsic function and regulation of the immune checkpoint regulator PD-L1 in HNSCC in order to explain treatment failures and to find new approaches for useful combination therapies.

Results: PD-L1 is expressed dependent on cell cycle progression. We demonstrated opposing cellular expression levels and localization of PD-L1, depending on their sensitivity to irradiation.PD-L1 total expression and localization changed significantly after irradiation. In radioresistant cell lines PD-L1 was stabilized due to inactivation of GSK3β. PD-L1 expression was influenced by PI3K/Akt signaling. PD-L1 has a strong impact on proliferation, migration and invasion as well as survival after irradiation.

Conclusion: PD-L1 function may not be generalized; rather, it is dependent on the particular cellular differentiation status and tumor microenvironment. Our data may provide an insight as to why differential radiosensitivity and response to PD-L1 antibody therapy occur during HNSCC treatment. The data showing the influence of PD-L1 on proliferation, migration, and invasion in HNSCC indicates the need for further investigation of PD-L1 regulation and its cellular functions besides its immunoregulatory effect. Detailed understanding of the various immune-independent cell-intrinsic PD-L1 functions, such as irradiation-associated localization and expression in different cellular compartments, will further allow antibody-based immunotherapy to be optimized.

P34

The prognostic impact of Axl and Gas6 in head and neck cancer

T. Rieckmann (Hamburg/DE), C. Hagel (Hamburg/DE), B. T. Becker (Hamburg/DE), A. Oetting (Hamburg/DE), N. Möckelmann (Hamburg/DE), C. S. Betz (Hamburg/DE), A. Münscher (Hamburg/DE), T. Clauditz (Hamburg/DE), C. J. Busch (Hamburg/DE, Greifswald/DE)

Introduction and objectives: The tyrosine kinase Axl is described as a driver for migration and metastasis as well as for resistance against molecular targeting agents as well as radio- and chemotherapy in various entities including HNSCC. For the latter, clinical data describing the impact of Axl and its ligand Gas6 on patient outcome are sparse. Therefore, we assessed the expression of both proteins and the association with patient survival in a single center retrospective cohort.

Materials and methods: Staining of Axl and Gas6 in a tissue microarray of a heterogeneous HNSCC cohort; classification of protein expression by manual inspection using an established algorithm; correlation with clinicopathological parameters; survival analyses.

Results: Overall 341 and 350 samples yielded interpretable staining for Axl and Gas6, respectively. Protein expression was neither correlated to T- or N-stage nor to the other protein. Axl expression levels had no significant impact on survival in patients with p16-positive oropharyngeal SCC (OPSCC). In a pooled cohort of patients with p16-negative OPSCC, laryngeal, hypopharyngeal or oral cavity SCC, largely comprising HPV-negative tumors, Axl expression did not demonstrate a significant impact in patients treated with adjuvant or primary radio(chemo)therapy. In patients treated solely with surgery, however, strong Axl expression was significantly associated with inferior overall and recurrence free survival (OS: p=0.0026; RFS: p=0.0056). In addition, Gas6 was a positive predictor of survival in patients whose treatment included radiotherapy (OS: p=0.0095; RFS: p=0.022). Both proteins remained independent predictors in multivariable analysis including T- and N-stage, sex and age.

Conclusion: Our data call into question the capacity of the Axl/Gas6 pathway to confer clinically meaningful radioresistance in HNSCC but rather suggest that strong Axl expression marks tumors that require adjuvant radio(chemo)therapy after surgery.

P35

Horizontal mitochondrial transfer from fibroblast to oral cancer cells promotes a stem cell-like and migratory phenotype

K. C. Debnath (Bergen/NO), R. Mustafa (Bergen/NO), H. Parajuli (Bergen/NO), S. Tornaas (Bergen/NO), D. Siyam (Bergen/NO), A. Kvalheim (Bergen/NO), D. E. Costea (Bergen/NO), H. Dongre (Bergen/NO)

Background: Cancer-associated fibroblasts (CAFs) are one of the most critical elements of the tumor microenvironment (TME) contributing to proliferation, invasion, and metastasis of oral squamous cell carcinoma (OSCC). However, the metabolic interaction between OSCC cells and the normal oral fibroblasts (NOFs) is not completely elucidated.

Aim: To identify the consequences of the metabolic coupling between OSCC cells and the surrounding fibroblasts from the TME.

Methods: NOFs were isolated from normal human oral mucosa after informed consent. NOFs" mitochondria were first tracked with fluorescent tags using DsRed lentiviral particle, then isolated using the commercially available mitochondrial isolation kit (ThermoFisher Scientific, USA). OSCC cells were isolated from primary HPV negative OSCC lesions after informed consent and "treated" with the isolated mitochondria. The effect of mitochondrial uptake on proliferation, stem cell-like properties and motility phenotype of recipient OSCC cells was investigated in vitro and vivo studies.

Results: Fluorescently tagged mitochondria isolated from NOFs were up-taken by OSCC cells at different rates, with the highest at 5µg concentration after 24 hours. OSCC cells treated with 5µg isolated mitochondria formed significantly higher number of colonies, bigger spheroids, and become more proliferative. In addition, they also invaded and migrated significantly more than the non-treated cells.

Conclusions: The results of this study show that the mitochondrial transfer from fibroblasts to OSCC cells confers to the latter both stem cell-like and migratory properties.

P36
Hyperspectral definition of head and neck tumor margins

H. N. Nguyen (Jena/DE), D. Pertzborn (Jena/DE), G. Ernst (Jena/DE), O. Guntinas-Lichius (Jena/DE), F. von Eggeling (Jena/DE), F. Hoffmann (Jena/DE)

Introduction: In the case of surgery for head and neck cancer (HNC) a complete removal is crucial for the outcome. However, highly accurate intra-surgical detection of tumor margins is currently not possible. Novel biophotonic imaging techniques such as hyperspectral imaging (HSI) allow for objective assessment of tumor margins. HSI is a non-invasive, non-ionizing, contrast agent free technique that combines spectroscopy with imaging. Indocyanine green (ICG) - a non-targeted near infrared fluorescent dye - can improve HSI results.

Objectives: This project aims to investigate the distribution of ICG in the microarchitecture of tumor margins in order to generate a spectral tumor signature with HSI technology. The long-term goal is to achieve better in-vivo determination of tumor boundaries.

Material and methods: Residual tumor material from 10 HNC patients who received ICG intravenously during surgery were used. Endoscopic and microscopic HSI setups were applied to investigate the boundaries between cancerous and non-cancerous tissue. Histological sections were examined to compare the distribution of ICG in cancerous and non-cancerous tissue regions.

Results: The results from the ICG studies were combined with those from HSI for a multimodal approach to enable a more accurate tumor margin detection. An experimental setup and workflow allowing standardized examination of tissues were developed and established. A spectral differentiation between different tissue regions was found to be possible. ICG was successfully detected within the tissue. Our findings correlated with corresponding histology.

Conclusions: HSI in combination with ICG can be used to examine tumor margins. These techniques will enable a more objective and accurate determination of tumor boundaries. For an intra-surgical in-vivo application in the future, a solid and valid spectral database has to be established.

Funded by Bundesministerium für Bildung und Forschung (BMBF; TheraOptik 3GW0370E)

P37

Validation of a classification and scoring system for the diagnosis of laryngeal and pharyngeal squamous cell carcinomas by confocal laser endomicroscopy

M. Goncalves (Aachen/DE), K. Mantsopoulos (Erlangen/DE), S. K. Mueller (Erlangen/DE), R. Rupp (Erlangen/DE), M. Eckstein (Erlangen/DE), F. Stelzle (Erlangen/DE), N. Oetter (Erlangen/DE), H. Iro (Erlangen/DE), M. Sievert (Erlangen/DE)

Introduction: Confocal laser endomicroscopy is an optical imaging technique that allows in vivo, real-time, microscope-like images of the upper aerodigestive tract's mucosa. The assessment of morphological tissue characteristics for the correct differentiation between healthy and malignant suspected mucosa requires strict evaluation criteria.

Objective: This study aims to validate an eight-point score for the correct assessment of malignancy.

Methods: We performed confocal laser endomicroscopy between March and October 2020 in 13 patients. 197 sequences (11.820 images) originated from the marginal area of pharyngeal and laryngeal carcinomas. Specimens were taken at corresponding locations and analyzed in H&E staining as a standard of reference. A total of six examiners evaluated the sequences based on a scoring system; they were blinded to the histopathological examination. The primary endpoints are sensitivity, specificity, and accuracy. Secondary endpoints are interrater reliability and receiver operator characteristics.

Results: Healthy mucosa showed epithelium with uniform size and shape with distinct cytoplasmic membranes and regular vessel architecture. Confocal laser endomicroscopy of malignant cells demonstrated a disorganized arrangement of variable cellular morphology. We calculated an accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of 83.2%, 81.3%, 85.5%, 86.7%, and 79.7%, respectively, with a κ-value of 0.64, and an area under the curve of 0.86.

Conclusion: The results confirm that this scoring system is applicable in the laryngeal and pharyngeal mucosa to classify benign and malignant tissue. A scoring system based on defined and reproducible characteristics can help translate this experimental method to broad clinical practice in head and neck diagnosis.

P38

Proteomes of exosomes from HPV(+) or HPV(-) head and neck cancer cells: differential enrichment in immunoregulatory proteins

S. Ludwig (Essen/DE), L. Marczak (Poznán/PL), P. Sharma (Pittsburgh, PA/US), A. Abramowicz (Gliwice/PL), M. Gawin (Gliwice/PL), P. Widlak (Gliwice/PL), T. L. Whiteside (Pittsburgh, PA/US, Pittsburgh, PA/US), M. Pietrowska (Gliwice/PL)

Question: Human papillomavirus (HPV) is an etiologic factor in head and neck squamous cell carcinoma (HNSCC). HPV(+) cancers respond favorably to therapy potentially due to more robust anti-tumor immune responses. We hypothesized that tumor-derived exosomes (TEX) produced by HPV(+) or HPV(-) HNSCCs differentially modulate anti-tumor immune responses.

Methods: Proteomes of exosomes from HPV(+) and HPV(-) HNSCC cell lines were compared in search for proteins putatively involved in the communication with immune system. TEX were isolated from supernatants of HPV(+) (SCC-2, SCC-47, and SCC-90) or HPV(-) (PCI-13 and PCI-30) cells by size exclusion chromatography. A comparison of proteome profiles was performed by high-resolution mass spectrometry. The presence and biological activity of selected immunoregulatory proteins were validated by flow cytometry and co-incubation assays.

Results: Exosomes produced by SCC-90 and PCI-30 cells contained 711 proteins, including 80 proteins specific for HPV(+) exosomes and 77 specific for HPV(-) exosomes, associated with similar GO terms such as regulation of cell growth, metabolism, communication, and cellular signaling. Search for proteins localized in the membrane and involved in immune regulation identified a few proteins detected specifically in HPV(+) or HPV(-) exosomes. Only HPV(+) exosomes were enriched in immune effector cell-related CD47 and CD276 antigens; only HPV(-) exosomes contained tumor-protective/growth-promoting antigens, MUC-1 and HLA-DA. Flow cytometry and Western blots confirmed the reciprocal presence/paucity of these proteins in a whole panel of tumor cells and corresponding exosomes.

Conclusions: The differential content of protein cargos in HPV(+) and HPV(-) exosomes might contribute to the disparity in immune responses that characterize HPV(+) and HPV(-) HNSCC.

P39

Inter- and intra-tumoral heterogeneity as drivers of radioresistance in HNSCC

S. Mutlu (Munich/DE, Heidelberg/DE), P. Branz (Munich/DE), G. Drexler (Munich/DE), O. Klymenko (Munich/DE), J. Hess (Neuherberg/DE), K. Unger (Neuherberg/DE), R. Astaburuaga (Berlin/DE), T. Sell (Berlin/DE), B. Klinger (Berlin/DE), N. Brix (Munich/DE), C. Belka (Munich/DE, Neuherberg/DE), N. Blüthgen (Heidelberg/DE, Berlin/DE), H. Zitzelsberger (Neuherberg/DE), K. Lauber (Munich/DE, Neuherberg/DE)

Introduction: Head and neck squamous cell carcinoma (HNSCC) still stands as a threat, being the sixth most common malignant tumor disease with limited therapeutic success. Multi-modality treatment of HNSCC involves surgery and/or radio(chemo)therapy. However, for locally advanced cases the 5-year overall survival rate remains below 50%.Inherent therapy resistance is considered as a major hurdle and is – to a remarkable extent – driven by inter- and intra-tumoral heterogeneity.

Objectives: We aim to provide insights into patterns of intrinsic radioresistance arising from inter- and intra-tumoral heterogeneity and to identify novel candidates for molecular targeting to improve the therapeutic outcome.

Materials and methods: Inter- and intra-tumoral heterogeneity in terms of clonogenic survival upon radiotherapy and different cell fate decisions were analyzed in panels of subclones established from the cell line Cal33 and from patient-derived explant cultures. Molecular correlates of intrinsic radioresistance were explored by integrating clonogenic survival data with RNA-Seq data**.**

Results: Our results show strong inter- and intra-tumoral differences in clonogenic survival upon ionizing radiation. Integration with transcriptomic data identified enriched genesets and signaling hubs involved in homologous recombination, the Fanconi anemia pathway, and nucleotide excision repair, whereas no association of inherent radioresistance with other classical DNA repair mechanisms, such as Non-homologous endjoining, were observed. In addition to DNA repair-related resistance, inter-tumorally we also observed other types of resistance patterns, including genesets associated with energy metabolism and cytokine regulation.

Conclusion: Our study reflects a substantial degree of heterogeneity in HNSCC on functional and molecular levels and suggests novel therapeutic vulnerabilities. These are currently being validated, with regard to both therapeutic performance and prognostic clinical value.

P40

Autophagy as a therapeutic target in head and neck squamous cell carcinoma

S. A. Kandathil (Vienna/AT), A. L. Lein (Vienna/AT), A. Scheiflinger (Vienna/AT), G. Heiduschka (Vienna/AT)

Introduction: Macroautophagy (hereafter referred to as "autophagy") is an evolutionarily conserved process to eliminate damaged proteins, protein complexes, and organelles in the cell. Therefore, the autophagic machinery facilitates cell survival under stressful circumstances, including tumor cells, which experience metabolic stress. However, the only FDA-approved drug to inhibit autophagy is Hydroxychloroquine (HCQ), but its use is limited due to its low potency and side effects at therapeutically relevant dosages.

Objectives: The objective of this study was to determine whether the novel autophagy inhibitors Lys05 and SBI-0206965 could sensitize more potently established head and neck squamous cell carcinoma (HNSCC) cell lines CAL27 and FaDu to the effects of Cisplatin and radiotherapy than HCQ.

Materials and methods: Cell viability and wound healing assay of the HNSCC cell lines CAL27 (tongue) and FaDu (hypopharynx) treated with the autophagy inhibitors Lys05 and SBI-0206965 only and combined with Cisplatin and/or radiotherapy were assessed. Additionally, synergistic or additive antitumor effects of the therapy combinations were assessed. Moreover, the effect on cell viability after treatment was also investigated in 3D spheroids comprised of CAL27 and FaDu grown in ultra-low attachment plates.

Results: Lys05 and SBI-0206965 showed an IC50 at 4uM, whereas the IC50 of HCQ varied between 20 to 60uM depending on the cell line. Cell migration was inhibited with novel drugs at doses ranging between 1 to 3uM. In comparison, HCQ could only inhibit cell migration at dosages between 10 and 50uM. Furthermore, chemotherapy - comprised of autophagy inhibition and Cisplatin - and radiotherapy combined showed a significant decrease in cell viability in 2D and 3D cell culture.

Conclusion: Chemically targeting autophagy with specific inhibitors in established HNSCC cell lines shows significant reduction of cell viability and impairment of cancer cell migration.

P41

Unraveling Druggable Key Nodes in Gene and Signaling Networks Related to Cancer-Neuron-Crosstalk in Head and Neck Squamous Cell Carcinoma (HNSCC)

K. Khorani (Heidelberg/DE), S. Burkart (Heidelberg/DE), J. Hess (Heidelberg/DE)

Introduction: Early in cancer development, nerve fibers (NF) form and infiltrate tumor tissue, and the density of NF in solid tumors has been associated with poor prognosis. Though the origin of cancer-related NF and the mode of their mutual interaction with cancer or stromal cells of the tumor microenvironment are elusive, NF and their associated cells are emerging as key regulators of cancer initiation, progression, and metastasis.

Objectives: (i) the clinical relevance of Schwann cells (SC) as a surrogate marker for peripheral nerve structures (PNS), as well as the molecular characterization of the cancer-nerve crosstalk (CNC) for HNSCC and other tumors. (ii) the establishment of preclinical models for new therapeutic strategies.

Material and methods: Publicly available multi-omics data for HNSCC and other tumor entities were collected and divided into training and validation cohorts. Via an integrative analysis, new models were predicted based on a SC-related gene signature. Models were evaluated using different computational and statistical models based on R-language programming and online web tools.

Results: The new model based on a SC-related gene signature in TCGA-HNSC was positively correlated with other PNS-related gene sets from public databases. Tumors with higher expression of the SC-related gene set shared a lower enrichment of immune cells and resembled an immune cold phenotype. Finally, these associations were confirmed in independent HNSCC cohorts and other tumor entities.

Conclusion: CNC represents a promising new avenue to establish more effective and less toxic strategies for early prevention or therapeutic intervention in HNSCC and other tumors. Our data indicate a potential role of SC in the modulation of the tumor immune phenotype, and ongoing studies aim to unravel the underlying molecular principles of gene-regulatory networks and oncogenic signaling pathways.

P42

Plasma-derived small extracellular vesicles (sEV) as potential biomarker of head and neck cancer (HNC)

M. Domnich (Essen/DE), E. Pylaeva (Essen/DE), E. Siakaeva (Essen/DE), I. Spyra (Essen/DE), I. Oezel (Essen/DE), I. Duerig (Essen/DE), M. Pietrowska (Gliwice/PL), S. Lang (Essen/DE), J. Jablonska (Essen/DE)

Introduction: Small extracellular vesicles (sEV) are membrane-bound vesicles of 30−150 nm in size that are secreted into the extracellular environment by all cells types, including cancer cells, and are responsible for carrying signal proteins between cancer cells and immune system. Identification of the unique cargo of sEV could help to understand mechanisms involved in cancer progression, supporting the development of novel biomarkers for HNC.

Material and methods: Size exclusion chromatography was used for isolation and purification of sEV from plasma samples of healthy donors (10) and HNC patients (34) with different cancer stages. The patients were classified according to UICC guidelines. Nanoparticle tracking analysis and western blotting were applied for exosomes characterization. Transmission electron microscopy was used for evaluation of heterogeneity and purity of exosomes. Global proteomic analysis of sEV was done to reveal their protein content. Statistical and bioinformatical analyses were performed using Perseus software.

Results: We have observed that the total protein content from sEV varies between a healthy group and cancer patients, and increases with the cancer stage. Proteomic analysis of plasma-derived exosomes led to identification of 99 proteins with significant changes in expression level between healthy and HNC patients. Most of them were represented in pathways connected to the regulation of immune system and hemostasis. Furthermore, hierarchical clustering of proteins revealed inter-related expression patterns and produced 7 clusters of highly related proteins that are differently regulated between high and low stages of HNC. Further investigations are needed to select target proteins for potential diagnostic and therapeutic approaches.

Conclusion: The differences in the expression pattern of proteins associated with plasma-derived sEV between healthy donors and cancer patients can be used as a potential HNC biomarker.

P43

Role of cancer stem cell markers ALDH1, BCL11B, BMI-1, and CD44 in the prognosis of advanced HNSCC

K. Sharaf (Munich/DE), J. Kitz (Göttingen/DE), C. Walz (Munich/DE), O. Gires (Munich/DE), M. Canis (Munich/DE), P. Baumeister (Munich/DE), M. Jakob (Munich/DE, Cologne/DE)

Introduction: To overcome stagnation in prognosis of HNSCC, research on the identification of prognostic markers and therapeutic targets is crucial. Cancer stem cells (CSCs) are hold accountable for the progress of head and neck squamous cell carcinoma (HNSCC).

Objectives: In the presented study, we evaluated the prognostic value of CSC markers in two particular HNSCC cohorts.

Methods: This two cohort study consisted of 85 patients with advanced stage HNSCC, treated with primary radio(chemo)therapy (pRCT), and 95 patients with HNSCC, treated with surgery and partially adjuvant radio(chemo)therapy. Overall survival (OS), disease free survival (DFS), and disease specific survival (DSS) were assessed. Samples were assessed for the expression of different molecular stem cell markers (ALDH1, BCL11B, BMI-1, and CD44).

Results: In the pRCT cohort, none of the baseline patient and tumor features exhibited a statistically significant relation with survival in both the cohort and HPV-stratified subcohorts. High expression of BMI-1 significantly decreased OS and DFS, while high expression of CD44 decreased all modes of survival. Multivariate analysis showed significant prognostic influence for all tested CSC markers, with high BMI-1 and CD44 decreasing survival (BMI-1: OS, DFS, DSS; CD44: OS, DFS) and high ALDH1, and BCL11B showing a beneficial effect on survival (ALDH1: OS, DFS; BCL11B: OS, DSS). In the surgical cohort, classical prognosticators such as HPV status, R1 resection, and nodal status in HPV negative HNSCC played a significant role but the tested CSC markers showed no significant influences on prognosis.

Conclusion: Though validation in independent cohorts is still needed, testing for CSC markers in patients with advanced or late stage HNSCC might be beneficial, especially if many comorbidities exist or disease is irresectable. The findings might guide the development and earlier use of targeted therapies in the future.

P44 - withdrawn

P45

Promising epigenetic biomarker for improvement of head and neck cancer diagnostic

A. B. Hums (Jena/DE), C. Dippmann (Jena/DE), A. Hennig (Jena/DE), L. Jansen (Jena/DE), M. Dürst (Jena/DE), M. Schmitz (Jena/DE), A. Hansel (Jena/DE), O. Guntinas-Lichius (Jena/DE)

Objectives: Two thirds of head and neck squamous cell carcinomas (HNSCC) are only diagnosed in advanced stages. Epigenetic changes in the DNA occur at an early stage in the tumorigenesis and may thus allow the development of diagnostics for early detection. Our aim is the development of an in vitro diagnostic test for early detection of HNSCC. Our current feasibility study OncSaliva aims to show that the epigenetic tumor marker ZNF671 can be detected in tissue and non-invasive specimen equally.

Methods: Analysis of ZNF671 (reference Beta-actin, ACTB) was performed using methylation-specific QPCR. Genomic DNA was isolated from each sample and bisulfite-converted prior to QPCR. The validation set included tissue from 63 HNSCC and 56 controls, as well as oral swabs from 31 HNSCC and 33 controls. So far, samples from 33 tumor patients and 7 controls were included in the study. All samples were collected in the Department of Otorhinolaryngology, Jena University Hospital.

Results: In the validation set ZNF671 yielded 71% clinical sensitivity (45 of 63) and 98% specificity (1 of 56) in tissue samples. In oral swabs ZNF671 showed slightly weaker detection, resulting in a clinical sensitivity of 65% (20 of 31) and a specificity of 88% (4 of 33). In pre-operatively collected saliva samples from the OncSaliva study ZNF671 was detected in 94% of the tumor samples (31 of 33). The clinical specificity within the control group was 71% in saliva (5 of 7) and 86% in the associated tissue samples (6 of 7). For 93% (25 of 27) of the tumor group and 71% (5 of 7) of the control group samples equal results for ZNF671 in saliva and tissue (both correspondingly positive or negative) were obtained.

Conclusion: Preliminary results from the feasibility study show that ZNF671 can be reliably detected in tissue, oral swabs and saliva. Therefore, the detection of ZNF671 in non-invasive samples may provide a useful diagnostic tool for secondary and tertiary prevention of HNSCC in the future.

P46

Exploitation of chromosome 11q13 alterations as therapeutic targets and predictive biomarkers in head and neck squamous cell carcinoma (HNSCC)

S. Bourdier (Berlin/DE), K. Riecken (Hamburg/DE), I. Tinhofer-Keilholz (Berlin/DE)

In HPV-negative HNSCC, 30% of patients harbor 11q13 amplifications in their tumors. Oncogenes present at this locus have been identified for contributing to a more aggressive tumor phenotype. Among these genes, cyclin D1 (CCND1), ANO1 and SHANK2 have been proposed as major oncogenic drivers in HNSCC that might contribute to the observed poor outcome of 11q13-amplified cases. The current project is therefore designed to dissect the individual role of 11q13 amplicon genes in tumor progression, their contribution to primary resistance to current treatments including radiation and cisplatin.

In the pilot phase of this project, the role of 11q13 gene amplifications was evaluated in a cisplatin-resistant subline of the HNSCC cell line FaDu harboring high-level CCND1 and ANO1 amplifications. Gene silencing of 11q13 amplicon genes by shRNA was combined with detailed genotype-phenotype analyses, including analysis of gene expression by qPCR and Western blot, and assessment of cell cycle distribution and apoptosis by flow cytometry. The interference of CCND1 and ANO1 knock-down (KD) with sensitivity to cisplatin treatment was determined using the MTT assay.

After confirmation of the KD on the transcriptomic and proteomic levels, phenotype analysis of the KD sublines was performed which revealed cell cycle arrest in G1 and strong induction of apoptosis upon CCND1 or ANO1 silencing. Treatment of selected KD clones with cisplatin showed a lower IC50 value compared to the control cells indicating resensitization of tumor cells toward platinum-based treatment by CCND1 / ANO1 targeting.

These findings confirm previous own data of a role of CCND1 and ANO1 amplification in cisplatin resistance. Treatment combinations with pharmacological drugs targeting CCND1/ANO1 expression could represent an attractive approach for resensitizing HNSCC tumors toward platinum-based therapy regimens. Currently, novel candidate drugs are being tested in HNSCC cell lines and organoid models.

P47

Role of platelets in tumor immune modulation via horizontal RNA transfer

M. Siegl (Munich/DE), M. K. Fritsche (Munich/DE), T. Weiser (Munich/DE), B. Wollenberg (Munich/DE)

Introduction: Platelets are small anucleated cells of the blood, mainly known for their function in hemostasis and thrombosis. Moreover, they play a crucial role in the immune response but also in cancer. Since platelets are known to form complexes with tumor, endothelial and immune cells, it is assumed that platelets are comprehensive effectors in tumor progression and immunity.

Objectives: This study should elucidate the horizontal gene transfer from tumor cells to platelets and if platelet RNA is passed on to a recipient cell. Cancer generic RNA profiles in platelets may then delineate them as possible immune modulators in tumors.

Materials and methods: Platelet RNA profiling of 55 patients with HNSCC and 17 healthy individuals was done by RNA-sequencing. Formation of platelet-leukocyte aggregates in whole blood was assessed by flow cytometry. In vitro transfer of SYTO™ RNASelect™ Green labeled RNA from tumor cells to isolated platelets was studied by flow cytometric analysis. Platelets were also transfected with eGFP-mRNA and after coincubation with PBMCs, fibroblasts or tumor cells the expression of eGFP in these recipient cells was evaluated by microscopy and/or flow cytometry.

Results: Platelet RNA profiles from HNSCC patients were different from healthy donors. Moreover, tumor platelets showed significantly more aggregates with monocytes, neutrophils and T-cells. RNA transfer from tumor cells to platelets could be observed in vitro. Vice versa, coincubation with eGFP mRNA transfected platelets did lead to eGFP expression in monocytes, fibroblasts and tumor cells.

Conclusion: Platelets are able to transfer RNA to a recipient cell. Given the differential RNA profile of platelets in HNSCC patients and enriched leukocyte-platelet aggregates, this implicates a role of platelets in tumor immune modulation via horizontal RNA transfer.

P48

PI3K/AKT/mTOR pathway and CDK4/6 inhibitors efficiently inhibit cell growth of HPV-positive and HPV-negative head and neck squamous cell carcinoma in vitro

I. Demers (Maastricht/NL), F. Verhees (Maastricht/NL), D. Legemaate (Maastricht/NL), R. Jacobs (Maastricht/NL), A. Hoeben (Maastricht/NL), F. Hoebers (Maastricht/NL), B. Kremer (Maastricht/NL), E. J. M. Speel (Maastricht/NL)

Introduction: Both HPV-positive and -negative head and neck squamous cell carcinoma (HNSCC) often show activation of the PI3K/AKT/mTOR pathway, due to, amongst others, PI3KCA mutations, PTEN loss, or receptor tyrosine kinases activation. In HPV-negative tumors, CDKN2A (p16) inactivation or CCND1 (cyclin D1) amplification frequently occurs resulting in sustained cyclin dependent kinase (CDK) 4/6 activation.

Objectives: The aim of our study was to investigate the efficacy of PI3K/AKT/mTOR pathway inhibitors (PI3Ki) (alpelisib, buparlisib and gedatolisib) and CDK4/6 inhibitors (CDKi) (palbociclib and ribociclib) in HPV-positive and -negative HNSCC cell lines.

Materials and methods: The efficacy of the inhibitors was assessed using MTT assays and changes in PI3K and Cyclin D1/CDK pathway protein expression were determined by Western blot. Cell cycle analysis was performed with flow cytometry and apoptosis was assessed by Annexin-V staining. Changes in cell metabolism were assessed by Seahorse XF assays.

Results: Both HPV-positive and -negative HNSCC cell lines were highly sensitive to the PI3Ki (Gedatolisib, IC50 of 5-30 nM > Buparlisib, IC50 of 0.6-3.6 µM > Alpelisib, IC50 of 3-23 µM). In general, PI3Ki decreased pathway activity, resulted in moderate cell cycle arrest and apoptosis and decreased oxidative and glycolytic metabolism. CDKi were particularly effective in blocking HPV-negative cell line growth (Palbociclib, IC50 of 0.5-2 µM > Ribociclib, IC50 of 4-7 µM). CDK inhibition showed decreased p-Rb expression and G1 cell cycle arrest, whereas apoptosis was not induced.

Conclusion: PI3Ki and CDKi efficiently inhibit their respective pathways and HNSCC cell growth in vitro, the latter only in HPV-negative cell lines. Whereas PI3Ki show a larger effect on oxidative and glycolytic metabolism, CDKi rather directly lead to cell cycle arrest. Further research should elucidate whether these inhibitors may be effective therapeutic agents in HNSCC patients.

P49

Targeted locus amplification-based sequencing for mapping viral integration sites in human papillomavirus positive head and neck squamous cell carcinomas

I. Demers (Maastricht/NL), H. Balaji (Cologne/DE), H. Feitsma (Utrecht/NL), I. Sergeeva (Utrecht/NL), J. Swennenhuis (Utrecht/NL), N. Würdemann (Cologne/DE), S. Wagner (Gießen/DE), B. Kremer (Maastricht/NL), C. Huebbers (Cologne/DE), J. P. Klußmann (Cologne/DE), E. J. M. Speel (Maastricht/NL)

Background: Human papillomavirus (HPV) infections are the principal cause of cervical cancers, subsets of anogenital and head and neck cancers (HNC). During persistent infection, viral DNA integration into the host genome may occur, which is suggested to affect carcinogenesis. One of the most critical limitations of currently used HPV integration detection techniques (PCR-based or NGS-based) is their application to FFPE material, because of the relatively short DNA fragments.

Objectives: The aim of this study was to assess HPV integration in HPV-positive HNSCC cell lines and FFPE tissue comparing the new Targeted Locus Amplification (TLA) technology with previously used PCR technology (APOT/DIPS).

Materials and methods: HPV-positive cell lines and FFPE material of HPV-positive HNSCC were used for HPV integration detection by TLA, a proximity ligation-based next-generation sequencing technique. Crosslinked DNA is digested with restriction enzymes, and re-ligated into chimeric DNA molecules. For cell lines, a PCR based HPV16 target enrichment is done, and for FFPE material a capture-based target enrichment is done for HPV16 and HPV18 sequences, both followed by Illumina sequencing.

Results: TLA was able to sequence up to 100 kb around the target, detecting exact HPV integration loci, structural variants, and chromosomal rearrangements. In all cell lines, one or more integration sites were identified, in accordance with APOT/DIPS PCR data and the literature. In the FFPE tissue samples, TLA identified either episomal or integrated HPV, in the latter case with simple and complex integration patterns. In general, TLA confirmed PCR data and detected additional integration sites.

Conclusion: TLA provides the opportunity for reliable and robust detection of HPV integration in HNSCC cell lines and FFPE tissue. This new sequencing technology could be a useful tool for further research on HPV integration in disease and patient outcome and eventually in clinical diagnostics.

P50

Measurement of oxygen content and consumption in primary tumors of the head and neck area via fluorescent sensor foil technology

M. Widmann (Munich/DE), G. Liebsch (Regensburg/DE), M. Canis (Munich/DE), A. Blancke-Soares (Munich/DE), O. Gires (Munich/DE), F. Haubner (Munich/DE)

Fluorescent sensor foil technology was used to evaluate oxygen content and consumption in human tumor tissue of the head and neck. Oxygen consumption was measured to differentiate between malignant and benign tissue.

In solid tumors, hypoxia is as a negative concerning therapeutic options. Especially with regard to poor response to irradiation therapy, hypoxia in tumor tissue has been the subject of previous and current research (1). High oxygen consumption enables the expression of transcription factors such as HIFs (hypoxia inducible factors) and signaling pathways that impact cellular functions such as apoptosis, necrosis, angiogenesis, and others (2). Hypoxia in tumor tissue is associated with poorer overall survival and poorer locoregional recurrence control (3).

A handheld device for recording data sets and special sensor foils with an oxygen permeable membrane and fluorescent dyes were used. Tumor lesions of the mouth, tongue and skin were chosen for analysis.

First *in vivo* measurements of tumor tissue of the tongue showed a significant difference of oxygen consumption in tumor tissue compared to healthy tissue. The oxygen consumption rate (OCR), was higher in tumor tissue than in healthy tissue. A differentiation between those two tissue types was made possible by analyzing the OCR.

Tissue oxygen content and consumption present as relevant parameters for the differentiation between healthy and tumor tissue of the head and neck region.

References

- Grimes, D. R., et al. 2017. Hypoxia imaging and radiotherapy. Bridging the resolution gap. *The British journal of radiology* 90,1076,20160939
- Brahimi-Horn, C., et al. 2006. The role of the hypoxia-inducible factor in tumor metabolism growth and invasion. *Bulletin du cancer* 93,8,E73-80
- Walsh, J. C. et al. 2014. The clinical importance of assessing tumor hypoxia. Relationship of tumor hypoxia to prognosis and therapeutic opportunities. *Antioxidants & redox signaling* 21,10,1516–1554

P51 - withdrawn

P52
Tracking clonal evolution in head and neck cancer

A. Pestana (Berlin/DE), B. Z. Peters Couto (Berlin/DE), K. Klinghammer (Berlin/DE), K. Riecken (Hamburg/DE), B. Fehse (Hamburg/DE), I. Tinhofer-Keilholz (Berlin/DE)

Multimodal regimens are in use for treatment of head and neck squamous cell carcinomas (HNSCC), however, long-term tumor control remains a challenge (1). Intratumoral heterogeneity (ITH) in HNSCC has been reported (2,3), and has been suggested as strong driver of therapy resistance and tumor recurrence (4,5). Here, we report on the exploitation of RGB marking of HNSCC organoid cultures to study ITH and clonal dynamics.

The FaDu cell line (representing a suitable HNSCC model for treatment-induced clonal evolution) (6) and an organoid culture established from a patient-derived xenograft (PDX) model of tongue cancer were used to establish the methods for RBG marking of HNSCC *ex vivo* models. Lentiviral vectors encoding for red (R), green (G) and blue (B) fluorescent proteins were used to generate cultures consisting of a large number of distinctly coloured clones (7). The clonal composition of the RGB marked cell line and organoid cultures was analysed by flow cytometry and confocal laser scanning microscopy, respectively.

Characterisation of RGB marked cultures revealed an influence of culture medium and extracellular matrix on size, shape and density of organoids. We also observed an interference of culture conditions with the clonality of individual organoids. Seeding of tumor cells in matrigel-coated plates and their culture in a medium optimized for epithelial organoids mainly resulted in monoclonal organoids (same colour hue). In contrast, seeding in polyhema-coated plates and/or the use of a culture medium for stem cells (StemXVivo) led to polyclonal structures, suggesting cell aggregation to so-called spheroids rather than organoid formation.

In summary, we demonstrate that RGB marking can be used for rapid, inexpensive and non-destructive analysis of clonal composition kinetics and spatial distribution in HNSCC organoids, rendering this approach a potent tool to study clonal dynamics, and to elucidate molecular mechanisms of therapy resistance in HNSCC.

P53

Interplay of oncogenic growth factor receptors and tumor-infiltrating immune cells in head and neck cancer

L. Fehr (Essen/DE), L. Kahl (Essen/DE), M. Schmidt (Essen/DE), J. Künzel (Regensburg/DE), S. Lang (Essen/DE), E. Deuss (Essen/DE), S. Brandau (Essen/DE)

Introduction: Head and neck squamous cell carcinoma (HNSCC) are a heterogeneous group of malignant tumors mainly related to HPV infections, tobacco and alcohol consumption. These risk factors can have an impact on the genomic profile of HNSCC classified by certain mutation patterns related to the cell cycle control and signaling pathways. The selected HNSCC show a strong expression of oncogenic growth factor receptors (GFR) of the EGF family. Oncogenes and the expression of GFRs may influence the immunobiology of the tumor and the inflammatory infiltrate.

Objectives: It is the aim of this study to determine the prognostic value of the inflammatory infiltrate in tumor core versus stromal regions of oropharyngeal squamous cell carcinoma (OPSCC) and to elucidate a potential interconnection of the tumor-infiltrating immune cells with the expression of GFRs.

Materials and methods: Formalin-fixed paraffin-embedded (FFPE) tissue samples of HNSCC patients were stained with cell markers via multiplex immunofluorescence and immunohistochemistry to visualize the immune infiltration of neutrophils, macrophages and T-cells and the expression of the GFRs EGFR, Her2-4 and c-Met in primary HNSCC and corresponding metastasis.

Results: Stromal regions of OPSCC showed a stronger infiltration with immune cells compared to tumor core regions. However, a higher density of tumor-infiltrating neutrophils (TAN) in the tumor core region was strongly correlated with poor survival. This was in contrast to densities of macrophages and T-cells that were not correlated with survival. Expression of ErbB2 correlated with higher densities of myeloid cells but not T-cells. Furthermore, TAN showed a statistically relevant correlation with high expression of EGFR.

Conclusion: Our data suggests a potential interconnection of EGF family members and neutrophil recruitment with consequences for tumor progression, treatment response and disease outcome.

P54

Location matters – head and neck cancers of different anatomical locations show distinct immune microenvironments

L. Kahl (Essen/DE), L. Fehr (Essen/DE), M. Schmidt (Essen/DE), J. Künzel (Regensburg/DE), S. Lang (Essen/DE), E. Deuss (Essen/DE), S. Brandau (Essen/DE)

Introduction: Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous tumor entity, which can be located in oropharynx (OPSCC), oral cavity (OSCC), larynx (LSCC) or hypopharynx (HPSCC). Laryngeal tumors are often diagnosed at earlier stages, whereas tumors in other anatomical sites are commonly recognized in advanced stages with regional lymph node metastasis. It is known, that many HNSCC tumors are infiltrated by immune cells, which can influence the prognosis of the patients.

Objectives: In this study the quantity, quality and spatial composition of the immune infiltrate in HNSCC was compared between the different anatomic locations and between primary tumors and lymph node metastases. We focused on the infiltration of neutrophils and macrophages as well as their interaction with cytotoxic T-cells.

Materials and methods: Multiplex immunofluorescence and immunohistochemistry staining of formalin-fixed paraffin-embedded (FFPE) tissue was assessed to analyze the tumor microenvironment (TME) in primary tumors and metastatic tumor lesions from HNSCC patients.

Results: A comparison of the TME in OPSCC, OSCC, LSCC and HPSCC primary tumors showed the strongest immune infiltrate in LSCC and the highest density of macrophages in OSCC tumors. Furthermore our data displayed a high density of neutrophils in stroma and a high density of T-cells in both tumor and stroma regions. Tumor islands of primary and metastatic OPSCC tumors exhibited a comparable density of myeloid cells but a different composition of T-cells. Metastatic lesions showed a higher density of immune checkpoint positive T-cells.

Conclusion: HNSCC from different locations display qualitative and quantitative differences in terms of their immune infiltrate, which should be considered when designing novel biological therapeutic regimens.

P55

HPV16-L1 antibody determination – evaluation of a new blood-based HPV tumor marker

T. Weiland (Graz/AT), P. Pondorfer-Schaefer (Graz/AT), P. Kiss (Graz/AT), S. Vasicek (Graz/AT), P. V. Tomazic (Graz/AT), A. Wolf (Graz/AT), U. Moser (Graz/AT), C. Holzmeister (Graz/AT), A. Andrianakis (Graz/AT), L. Brcic (Graz/AT), D. Thurnher (Graz/AT)

Question: The incidence of HPV16-induced oropharyngeal carcinomas has been shown to increase in industrialized countries and now exceeds that of cervical carcinomas in the USA, for example. A multicenter study has already shown the potential of a novel blood-based assay for the detection of relevant HPV16 antibodies in oropharyngeal cancer patients. In a follow-up study, the possible use as a post-treatment biomarker is to be examined in particular.

Material and methods: In a non-interventional, prospective study, 41 patients with initially diagnosed oropharyngeal cancer have been analyzed for the presence of antibodies against HPV16-L1 using a further developed assay. This was done before, during and after therapy with a follow-up for up to 3 years. Biopsies were also checked for HPV DNA and p16 expression.

Results: 25 HPV16-DNA positive patients showed significantly higher antibody titers compared to 16 HPV16-negative patients. An AUC of 0.91 was calculated using the ROC analysis of the HPV16-induced vs. HPV16-negative OPSCC. Using the Youden Index, the optimal cut-off of the test was determined to be 961 ng / ml to differentiate HPV16 positive from HPV16 negative OPSCCs, which resulted in a sensitivity of 72% and a specificity as well as a positive predictive value of 100%.

The majority of HPV16-DNA / p16 positive patients showed a "classic" course with falling antibody titers under tumor-specific therapy and constant "basal" titers in the follow-up. In two cases to date, a tumor recurrence could even be proven serologically before clinical diagnosis.

Conclusion: Based on the performance data calculated in our collective, the immunoassay appears to be a promising instrument for identifying relevant HPV16-related diseases. The test could be of great use in the future, particularly for measuring the success of the therapy and the early detection of recurrences in the follow-up.

P56

Correlations between pathological parameters and mutations detected by targeted next-generation sequencing of cancer-related genes in a Norwegian patient cohort with head and neck squamous cell carcinoma

H. Dongre (Bergen/NO)

Background: The mutational landscape of head and neck squamous cell carcinomas (HNSCC) has been described, however, correlations with pathological parameters have not been so far investigated. Targeted next-generation sequencing (NGS) is increasingly applied in both research and clinical oncology to advance personalized treatment. However, the use of targeted NSG panels alone or in combination with pathological parameters for assisting diagnosis and treatment of (HNSCC) is still limited.

Aim: The focus of this study was to investigate correlations of specific mutational landscapes with clinical and pathological parameters in a cohort of HNSCC.

Materials and methods: Tumour DNA obtained from archival FFPE tissue blocks of HNSCC patients treated at Haukeland University Hospital between 2003-2016 (n=111) was subjected to mutational analysis using a custom made AmpliSeq library plus panel targeting 32 genes (Illumina). Year- and side-matched normal samples (n=9), samples with known specific mutations, and Acrometrix oncology hotspot control (Thermo Fisher Scientific) were used for quality control of the NGS panel. Associations between mutational burden and clinical and pathological parameters were investigated using SPSS.

Results: In HPV-negative HNSCC mutations were detected mainly in TP53 (73.3%), FAT1 (26.7%) and FLG (16.7%) whereas in HPV-positive HNSCC the common mutations were in FLG (24.3%), FAT1 (17%) and FGFR3 (14.6%) genes. The presence of one or more cancer-specific mutations was found positively associated with a more aggressive type of invasive front (p=0.035), a richer desmoplastic stroma (p=0.019), and negatively associated with the degree of differentiation (p=0.041).

Conclusion: Novel associations between mutational burden and pathological parameters were detected.

P57

High hydrostatic pressure devitalizes head and neck cancer cells and induces immunogenic cell death

S. Daniel (Rostock/DE), T. Kleitke (Rostock/DE), H. Braunschweig (Rostock/DE), N. Schoenwaelder (Rostock/DE), F. Poosch (Rostock/DE), R. Mlynski (Rostock/DE), C. Maletzki (Rostock/DE)

Introduction: Immune checkpoint inhibition is a milestone in the therapy of advanced HNSCC. Nevertheless, many patients do not benefit from immune checkpoint modulation alone or in combination with irradiation and chemotherapy. One promising approach to reactivate immunosurveillance and extend immunotherapy is the development of therapeutic anti-tumor vaccines. However, head and neck cancer vaccines have not yet presented their true potential in clinical trials.

Objective: We introduce high hydrostatic pressure (HHP) for safe head and neck cancer devitalization and induction of immunogenic cell death. HHP specifically devitalizes cells without major changes in molecular structures. Therefore, HHP may enhance the development of whole-cell anti-tumor vaccines, which represent tumor heterogeneity and thus antigen-diversity.

Materials and methods: Cell lines (UTSCC14, HNSCC16 P1 M1, HNSCC46 P0 M2) were treated with 150, 300, and 450 MPa HHP for 10 minutes. Cytotoxicity was measured by crystal violet staining and flow cytometric apoptosis/necrosis discrimination. Immunogenic cell death was studied by flow cytometry of Calreticulin, ELISA of HMGB1, and luminescent ATP detection. HE-Histology after implantation of 1x105 cancer cells on the chicken chorion allantois membrane was used to confirm cell devitalization *in vivo*.

Results: We found in controlled *in vitro* assays that the presence and type of cell death depend on the HHP amplitude: 150 MPa HHD led to incomplete apoptotic cell death while HHP above 350 MPa induce complete and predominantly necrotic cell death. Cell death was immunogenic as increasing HHP triggered Calreticulin translocation to the surface and ATP/HMGB1 release. We confirmed the complete devitalization of head and neck tumor cells *in vivo* by excluding growth in the chorion allantois membrane assay.

Conclusion: Our results show that HHP based autologous whole-cell anti-tumor vaccines may complement immunotherapy combinations for high mutational burden cancer.

P58
Early recurrence detection through liquid biopsy in HNSCC

R. Roesch (Munich/DE), I. Kerle (Munich/DE), N. Pfarr (Munich/DE), K. Steiger (Munich/DE), F. Stögbauer (Munich/DE), J. Rinecker (Munich/DE), S. Krippgans (Munich/DE), C. Straube (Munich/DE), M. Nieberler (Munich/DE), W. Weichert (Munich/DE, Heidelberg/DE), J. Ruland (Munich/DE, Heidelberg/DE), B. Wollenberg (Munich/DE, Heidelberg/DE), C. Winter (Munich/DE, Heidelberg/DE), M. Wirth (Munich/DE, Heidelberg/DE)

Introduction: The examination of body fluids for tumor components (liquid biopsy) could enable an earlier detection of recurrences in the follow-up care of patients with squamous cell carcinoma of the head and neck (HNSCC). The aim of the study was to analyze the early recurrence detection by means of personalized, tumor-specific ddPCR mutation assays in blood and saliva.

Objective: To evaluate the feasibility of early recurrence detection with liquid biopsy in head and neck cancer in both blood and saliva.

Materials and methods: Prospectively, blood and saliva samples were collected from 8 HNSCC patients. Saliva samples were also taken from 7 patients in the postoperative follow-up, 7 plasma samples were available preoperatively. Panel sequencing (45 genes, 224 amplicons) identified mutations in the primary tumor. Based on this, patient-specific ddPCR mutation assays were designed and ctDNA in plasma and saliva was analyzed. At least 2 mutation-specific copies were defined as positive ctDNA detection.

Results: Using mutation-specific ddPCR assays, ctDNA could be detected in 71% (5/7) of pretherapeutic plasma samples. In the follow-up probes, ctDNA could be measured in 100% (7/7) of saliva and in 50% (4/8) of plasma probes. 5 patients developed a tumor relapse, 3 patients are clinically tumor-free. The ctDNA detection was associated with recurrent disease in 80% in the plasma and in 100% in the saliva. Liquid profiling showed a median increase in ctDNA in plasma 5.5 months (range: 1-20 months) and in saliva 7.0 months (range: 1-15 months) prior to the clinical diagnosis of recurrence.

Conclusion: The detection rate of ctDNA was higher in saliva. Saliva appears to be especially suited for the early recurrence detection of tumors of the oral cavity and oropharynx.

P59

Differential stroma-mediated drug sensitivity of oral squamous carcinoma cells

R. Mustafa (Bergen/NO), S. Tornaas (Bergen/NO), H. Parajuli (Bergen/NO), A. Kvalheim (Bergen/NO), B. Ljøkjel (Bergen/NO), K. M. Hoven (Bergen/NO), H. Dongre (Bergen/NO), D. E. Costea (Bergen/NO)

Introduction: Several recent studies have demonstrated a stroma-mediated drug resistance in malignant solid tumors. The context of tumor stroma and its effect on drug sensitivity of cancer cells is variable and depends on multiple factors such as tissue of origin, genetic alterations of cancer cells, progression stages, *etc*.

Aim: To investigate the potential of normal oral fibroblasts (NOF) *vs*. cancer associated fibroblasts (CAF)-containing tumor microenvironments to modulate drug resistance of oral squamous cell carcinoma (OSCC) cells.

Materials and methods: A panel of OSCC cells lines (UK1, OSCC1, Ca1) were seeded in 2D and 3D cultures in either monoculture or in co-culture with primary NOF and CAF isolated from OSCC lesions. Various concentrations of 5-Fluorouracil (5-FU) were added to medium on day 3 and cell viability (Resazurin) was assessed for five consecutive days.

Results: When cultured in 2D in monoculture, OSCC cells showed a dose-dependent decrease in cell viability when treated with 5-FU. When co-cultured with CAF, the percentage of viable cells increased significantly, *e.g.* from 37.9±2.2% to 51.1±10.1% for UK1 cell line when treated with 2.5µM 5-FU. A significant increase in cell viability was also observed when OSCC cells were grown in 3D compared to 2D, both in monocultures and in co-cultures with fibroblasts. No effect on cell viability was observed when OSCC cells were co-cultured in 3D together with CAF and treated with a wide range of concentrations of 4-FU (1µM to 300µM). In contrast, cell viability was decreased from *e.g.* 84±6.05% to 41.8±10.5% when OSCC1 cells were co-cultured in 3D with NOF and treated with same range of 5-FU concentrations.

Conclusions: Significant decrease in the drug sensitivity of OSCC cells was observed in 3D compared to 2D models and in mono-cellular cultures compared to co-cultures with CAF. 3D co-culture of OSCC with NOF partially restored the drug sensitivity of OSCC cells.

P60

Interaction studies between oral cancer cells and induced pluripotent mesenchymal stem cells (iPSMSC) reveal a complex cross talk leading to increased tumorigenesis

D. Siyam (Bergen/NO), H. Parajuli (Bergen/NO), H. Dongre (Bergen/NO), H. Mohammed (Bergen/NO), T. Osman (Bergen/NO), H. Ræder (Bergen/NO), K. Mustafa (Bergen/NO), D. E. Costea (Bergen/NO)

Introduction: Induced pluripotent mesenchymal stem cells (iPSMSC) hold the promise for a novel, more efficient therapy for many diseases, including cancer and disorders of the immune system. However, due to their immunomodulatory properties, the cross-talk with cancer cells should be critically studied before clinical use.

Methods: iPS reprogrammed from adult human normal oral fibroblasts (NOF) were differentiated into iPSMSC and co-cultured with OSCC-derived cell lines. Cell proliferation, cell migration and 3D organotypic (3D OTs) assays were performed to identify changes in proliferation, migration and invasion, respectively. Further, nine NOD/SCID IL-γ2-deficient mice with tongue xenografted with human OSCC cells were randomly divided into three groups and intravenously (IV) injected with: A) 1*106 iPSMSC (n=4), B) 1*106 matching NOF (n=4), and C) control (n=1), which did not receive any cells in the tail vein. Human cells were detected by dual immunohistochemistry for detection of human vimentin and mutated p53.

Results: Condition media from cocultured iPSMSC and OSCC increased proliferation, whereas no significant difference was observed in the migration of OSCC cells. In 3D OTs, OSCC cells invaded more in the underlying matrix populated with matched NOFs as compared to matrix populated with iPSMSCs. The tumour size increased in mice injected with iPSMSC as compared to both matched NOFs and controls. Interestingly, we could observe "homing" of both iPSMSC and NOF to the tongue tumours after 1 week of IV injections. In addition, lung metastases were found in mice with iPSMSC and NOF IV injections after one week.

Conclusion: IV administered iPSMSC homed to OSCC xenografts in tongue, increased tumour size and facilitated lung metastases within a week in the immunodeficient mice. More investigations are required to completely understand the iPSCMSC-OSCC cross-talk and its role in tumour progression.

P61

Frequent PD-L1 expression in head and neck squamous cell carcinoma of never-smokers and never-drinkers, and association of CD45 and CD8 positive tumor infiltrating lymphocytes with favorable survival

F. Mulder (Maastricht/NL), E. de Ruiter (Utrecht/NL), T. Gielgens (Maastricht/NL), F. Farshadpour (Amsterdam/NL), R. de Bree (Utrecht/NL), M. van den Hout (Maastricht/NL), B. Kremer (Maastricht/NL), S. Willems (Utrecht/NL, Groningen/NL), E. J. M. Speel (Maastricht/NL)

Introduction: Recently, expression of programmed death ligand 1 (PD-L1) was implemented as a predictive biomarker for immunotherapy in head and neck squamous cell carcinoma (HNSCC).

Objectives: The goal of this study was to determine if in HNSCC of non-smokers and non-drinkers (NSND) PD-L1/2 expression and tumor infiltrating lymphocytes (TILs) are present, and have prognostic value.

Materials and methods: Clinical data and tumors of 114 NSND HNSCC patients were collected retrospectively. Immunohistochemistry was performed for PD-L1, PD-L2, and lymphocyte markers PD-1, CD45, and CD8 on Tissue MicroArrays. Difference in 5-year survival of patients with and without expression of these biomarkers was determined using Kaplan-Meier and log rank analysis.

Results: The patients were on average 71.6 years old and predominantly women (77%) with a tumor of the oral cavity (78%). PD-L1 (tumor proportion score ≥1% and ≥50%) and PD-L2 (≥1%) expression was detected in 70%, 18%, and 10% of the tumors, respectively. A high number of CD45 and CD8-positive TILs (≥150/mm2) was a predictor for a significantly better 5-year disease free (HR CD45=.50, *p*=.010; HR CD8=.51, *p*=.013) and overall survival (HR CD45=.40, *p*=.001; HR CD8=.50, *p*=.014). This significant difference persisted, regardless of which cut-off value for TILs/mm2was applied (<150 TILs/mm2, 150-500 TILs/mm2, or >500 TILs/mm2).

Conclusion: PD-L1 expression is present in a large percentage of HNSCC in NSND. This might have implications for the application of immunotherapy in this patient group. A high number of CD45 and CD8-positive TILs is predictive for a better disease free and overall 5-year survival.

P62

Development of an endoscope-based plasma source and new applications of cold atmospheric plasma (CAP) in head and neck surgery

C. Scharf (Greifswald/DE), M. Wilhelm (Greifswald/DE), C. Scheffler (Greifswald/DE), M. Dib (Greifswald/DE), C. J. Busch (Greifswald/DE)

Background: Over the past decade, cold atmospheric plasma (CAP), a near room temperature ionized gas has shown its promising application in cancer therapy. In contrast to conventional anti-cancer approaches and drugs, CAP is a selective anti-cancer treatment modality. Previous experimental studies have demonstrated that CAP treatment results in significant growth inhibition of tumor cells and is able to induce apoptosis. In contrast to existing plasma sources, a new endoscope-based plasma source first had to be developed for applications in head and neck surgery.

Methods: Cold atmospheric plasma (CAP) was generated via a new developed endoscope-based atmospheric pressure plasma jet (PLASMASCOP). Physiological and molecular effects of CAP treatment on various human head and neck squamous cell carcinoma (HNSCC) cell lines compared to human epithelial cell lines were analyzed by cell counting, FACS analysis, COMET-Assays, Proteome and Western blot analyses.

Results: CAP treatment effectively attenuates malignant cell growth. The results demonstrated that even a single application of a short-term CAP treatment led to an attenuation of HNSCC cell growth and motility. We studied the detailed cellular adaptation reactions for a specified plasma intensity by time-resolved comparative proteome analyses of CAP treated vs. nontreated cells to elucidate the molecular mechanisms and to define potential biomarkers and networks for the evaluation of plasma effects on human tumor cells. Results were consistent in various HNSCC cell lines.

Conclusion: In summary, the CAP application in head and neck surgery leads to strong anti-proliferative effects and opens up novel opportunities for the treatment of HNSCCs. As an intraoperative application, CAP may represent a promising option particularly for the treatment of tissue regions that are close to critical structures (e. g. nerves, adjacent organs).

P63

Trophoblast cell surface antigen 2 (Trop-2) protein is highly expressed in salivary gland carcinomas and represents a potential therapeutic target

P. Wolber (Cologne/DE), L. Nachtsheim (Cologne/DE), F. Hoffmann (Cologne/DE), J. P. Klußmann (Cologne/DE), M. Meyer (Cologne/DE), F. von Eggeling (Cologne/DE), O. Guntinas-Lichius (Cologne/DE), A. Quaas (Cologne/DE), C. Arolt (Cologne/DE)

Treatment options for unresectable, recurrent or metastatic salivary gland carcinomas (SGC) are scarce. Trophoblast cell surface antigen 2 (Trop-2) is a transmembrane glycoprotein that is involved in a variety of oncogenic cell signaling pathways. Its potential as a target for the antibody-drug conjugate sacituzumab govitecan has already been demonstrated in different tumor entities. The United States Food and Drug Administration approved this antibody-drug conjugate for the treatment of metastatic triple-negative breast cancer. Here, we aimed to investigate Trop-2 protein expression in different entities of SGCs.

We retrospectively reviewed the medical records of all patients that underwent surgery for a primary SGC in a tertiary referral center between 1990 and 2014. Immunohistochemical (IHC) staining for Trop-2 was performed and rated as negative, weak, moderate or high using a semiquantitative score. Additionally, representative cases were analyzed using MALDI-mass spectrometry (MS) imaging to confirm the IHC results.

The cohort consisted of 114 tumors of the parotid gland (90.4%) and submandibular gland (9.6%). It mainly included mucoepidermoid, salivary duct and adenoid cystic carcinomas. In IHC samples, 44% showed high, 38% moderate and 10% weak expression rates of Trop-2. MALDI-MS imaging confirmed the presence of Trop-2 protein in 80% of the tested tumor samples.

This is the first study to demonstrate that several types of SGC express Trop-2 with variable intensity. Since there are currently few systemic treatment options for advanced SGCs, Trop-2 represents a promising target for further clinical studies, for instance, with sacituzumab govitecan.

P64

DNA methylation landscape of epidermal growth factor receptors (*EGFR*, *ERBB2*, *ERBB3*, *ERBB4*) in head and neck squamous cell carcinoma

J. Gabrielpillai (Bonn/DE), Y. Bao (Bonn/DE), R. Zarbl (Bonn/DE), S. Strieth (Bonn/DE), D. Dietrich (Bonn/DE)

Introduction: The monoclonal antibody cetuximab blocks the epidermal growth factor receptor EGFR (ERBB1) and is approved for the treatment of HNSCC. However, the low response rate to cetuximab (approx. 5-15%) necessitates the development of companion predictive biomarkers for upfront treatment decisions. DNA methylation is an epigenetic mechanism of high relevance in oncogenesis and DNA methylation testing represents a promising approach for biomarker development and there is no conclusive data on the role of DNA methylation of ERBBs in cancer.

Material and methods: We performed comprehensive correlative analyses of DNA methylation of *EGFR*, *ERBB2* (*HER2*), *ERBB3*, and *ERBB4* at single CpG site resolution with regard to gene (mRNA) expression, human papillomavirus (HPV) status, gene amplification, and response to the EGFR-targeted antibody cetuximab and small molecules (tyrosine kinase inhibitors) gefitinib and afatinib. We analyzed the HNSCC cohort included in The Cancer Genome Atlas (TCGA) and cell lines included in the Genomics of Drug Sensitivity in Cancers (GDSC) project.

Results: For all analyzed genes, we found a sequence-contextually nuanced CpG methylation landscape. DNA methylation was strongly different in tumor tissue compared to normal adjacent tissue. For *EGFR* and *ERBB4*, mRNA expressions were correlated to DNA methylation in tumor tissue for most of the analyzed CpG sites. For *EGFR*, we found a strong association of DNA methylation with gene amplification. We observed a significant CpG-dependent association of DNA methylation with HPV status for all investigated genes. Additionally, we elucidated significant, mostly positive, correlations of DNA methylation with response to cetuximab, gefitinib, and afatinib.

Conclusion: DNA methylation is involved in gene regulation and expression of *EGFR* and *ERBB2-4* in HNSCC. Our data provide a rationale for further testing DNA methylation as predictive biomarker parameter for response to EGFR inhibitors in clinical trials.

P65

Telomere length screening in young patients with head and neck cancer is suitable to detect underlying inherited telomere biology disorders (part of the work will be presented at the DGHO annual meeting 2021)

M. Tometten (Aachen/DE), B. Rolles (Aachen/DE), B. J. Siegmund (Aachen/DE), M. Klein (Aachen/DE), M. Ooms (Aachen/DE), P. Winnand (Aachen/DE), E. Simons (Aachen/DE), M. Bourgeois (Aachen/DE), G. Spanier (Regensburg/DE), S. Laban (Ulm/DE), M. Weber (Erlangen/DE), C. Goetz (Munich/DE), F. Birkenfeld (Kiel/DE), K. Kricheldorf (Aachen/DE), S. Isfort (Aachen/DE), M. Kirschner (Aachen/DE), S. Hackenberg (Aachen/DE), F. Hoelzle (Aachen/DE), F. Beier (Aachen/DE), T. H. Bruemmendorf (Aachen/DE)

Question: Head and neck (HN) cancer affects males in 6th to 7th decade. Major extrinsic risk factors (RF) are alcohol, tobacco and HPV-infection. A subset is at young age <50years (y). Telomere biology disorders (TBD) are characterized by impaired telomere maintenance. TBD patients (pts) are predisposed for solid cancers at early age, HN cancer represents the most frequent entity. Functional TBD read-out is premature shortened telomere length (TL). We systematically analyzed TL in a young HN cancer cohort to identify underlying TBD cases.

Methods: All pts were included in the *Aachen TBD registry* based on clinical suspicion for TBD. Pts ≤50y at initial cancer diagnosis with or without missing RF were eligible. Lymphocyte (L) and granulocyte (G) age-adjusted (aa) TL were analyzed by flow-FISH, next generation sequencing (NGS) for telomere maintenance gene mutations performed in cases with TL below the 10th percentile.

Results: Six German centers included 40 pts in this ongoing study. Mean age at initial diagnosis was 42.0 ± 7.2y. Major site and histology was oral tongue and squamous cell cancer, respectively. Tumor stage and RF available in 29 pts showed 18 (62%) with stage III or IV, smoking in 13 (45%), alcohol abuse in six (20%) and 10 (34%) with no history for both RF. HPV/p16 status was negative in all analyzed cases (15/15). AaTL in L was significantly lower (-0.59 (p= 0.017)) compared to healthy controls, while aaTL in G was normal (0.45 (p= 0,059)) representing premature aging. TL in L was below the 10th percentile in five pts, in two pts below the 1st. NGS screening revealed variants in two pts affecting the telomere maintenance genes CTC1 and NHP2, classified as variant of uncertain significance and likely pathogenic, respectively.

Conclusion: TL screening in young HN tumor pts can identify underlying inherited TBD and might improve clinical management. Further studies are needed to address the role of premature TL shortening in this pre-selected group.

P66

Analysis of oxidative stress related HPV16-E6*I expression in HPV-positive squamous cell carcinoma of the oropharynx

H. Balaji (Cologne/DE), N. Würdemann (Cologne/DE), I. Demers (Maastricht/NL), O. Seifer (Cologne/DE), J. George (Cologne/DE), E. J. M. Speel (Maastricht/NL), J. P. Klußmann (Cologne/DE), C. Huebbers (Cologne/DE)

Introduction: Cancer cells of head and neck squamous cell carcinoma (HNSCC) associated with high-risk types of human papillomavirus (HR-HPV) display altered energy metabolic pathways due to numerous oncogenic events activated by viral oncoproteins (E6 and E7) and their splice variants. E6*I is one of the major splice variants that is considered to be involved in both metabolic reprogramming and increased reactive oxygen species (ROS) metabolism, which further corresponds to unfavorable survival.

Objectives: We analyzed for E6*I expression in HNSCC cell lines and generated cell lines overexpressing HPV16-E6*I-His-GFP as a model to study this splice variant. Further, the metabolic and ROS profiles are determined in transfected and HNSCC cell lines.

Material and methods: HEK293 cells and primary keratinocytes are transfected with His-GFP tagged E6*I. Moreover, RT–qPCR assays are carried out to check mRNA expression of E6, E7 and splice variants. Ideal cell lines in addition to the transfected cell lines are chosen for subsequent assays. Life-cell assays are performed to analyze the changes in metabolic rates and ROS activity.

Results: His-GFP tagged, E6*I transfected HEK293 cells and keratinocytes are produced. Cell lines CaSki and UM-SCC-90 show significant expression of splice variant E6*I compared to other cervical and HNSCC cell lines (SiHa, UM-SCC-47, 93-VU-147T, UD-SCC-2, UPCI-SCC-090 and UM-SCC-104). Moreover, metabolic rates and ROS activity are correlated with E6*I expression.

Conclusion: HPV16-E6*I expression varies in HNSCC cell lines and further correlates with varying levels of ROS and metabolic rates. Therefore, determining the E6*I expression level might be of interest for subsequent analysis of ROS related and metabolic vulnerabilities of HPV-positive HNSCC. This could be interesting for new treatment options for a E6*I overexpressing subgroup of HNSCC corresponding to unfavorable survival.

P67

Risk stratification in head and neck cancer patients undergoing radiotherapy using 2-[18F]FDG PET/CT and artificial neural networks

S. Marschner (Munich/DE), E. Lombardo (Munich/DE, Garching/DE), L. Minibek (Munich/DE), A. Holzgreve (Munich/DE), L. Kaiser (Munich/DE), N. L. Albert (Munich/DE), C. Kurz (Munich/DE, Garching/DE), M. Riboldi (Garching/DE), R. Späth (Munich/DE), P. Baumeister (Munich/DE), M. Niyazi (Munich/DE), C. Belka (Munich/DE), S. Corradini (Munich/DE), G. Landry (Munich/DE, Garching/DE), F. Walter (Munich/DE)

Question: In this analysis, we retrospectively evaluated the performance of artificial neural networks (ANN) for predicting overall survival (OS) or locoregional failure (LRF) in head and neck cancer patients based on 2-[18F]FDG-PET/CT features and various clinical covariates.

Methods: We evaluated predictions on the basis of three different feature sets extracted from 230 patients undergoing radiotherapy. In particular, we compared (i) an automated feature selection method independent of expert judgment with (ii) clinical variables with proven impact on OS or LRF and (iii) clinical data plus expert-selected SUV metrics. These three sets of different variables served as input to an artificial neural network. Outcome prediction was evaluated using the Harrell"s Concordance Index (HCI) and by testing stratification ability.

Results: The best performance for OS and LRF was achieved with expert-based PET features (0.71 HCI) and clinical variables (0.70 HCI), respectively. Although only the expert-based PET features successfully classified patients into low and high risk for LRF, all three feature sets were significant for OS stratification as shown in figure 1.

Conclusion: Patient stratification into different risk groups for OS and LRF is possible using artificial neural networks based on 2-[18F]FDG PET/CT features and clinical covariates. The variations in the results for the three different feature sets confirm the importance of the feature selection process and expert knowledge compared to automatic selection.

Figure

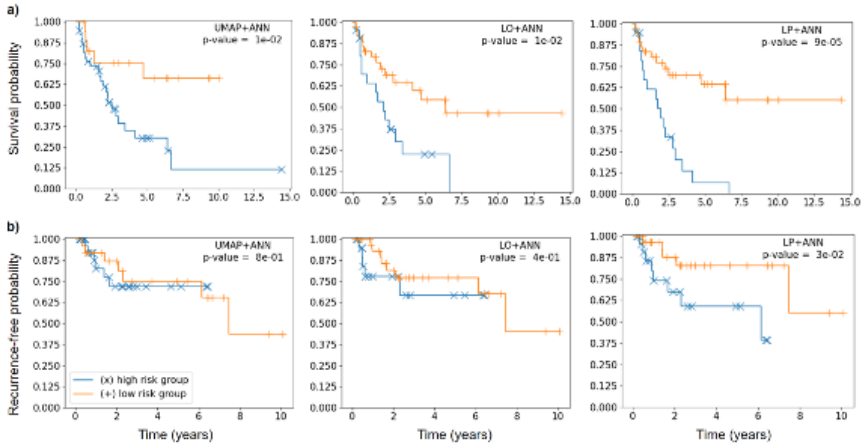


Figure 1. Kaplan-Meier curves of high-risk (blue) and low-risk (orange) patient groups separated according to a threshold optimized during cross-validation. Significance of difference was assessed using the log-rank test. (a) Kaplan-Meier plots for OS endpoint. (b) Kaplan-Meier plots for LRF endpoint.

P68

Anti-tumoral properties of neutrophils are activated by type I IFNs already during granulopoiesis

E. Siakaeva (Essen/DE), I. Spyra (Essen/DE), E. Pylaeva (Essen/DE), M. Domnich (Essen/DE), A. Hasenberg (Essen/DE), S. Lang (Essen/DE), J. Jablonska (Essen/DE)

Introduction: Tumor-driven immune response plays a controversial role in cancer development. Thus, tumor-associated neutrophils (TANs) are able to exert pro- or antitumoral activity depending on activation stimuli. Type I interferons (IFNs) were shown to support anti-tumoral bias of neutrophils. Moreover, type I IFNs also influent neutrophil development (granulopoiesis) in hematopoietic organs.

Objectives: We aimed to analyze tumor-driven granulopoiesis in IFN-deficiency to assess whether type I IFNs modify anti-tumoral properties of neutrophils already during their development in hematopoietic organs.

Materials and methods: We have compared the course of granulopoiesis in WT and type I IFN-deficient *(Ifnar1-/-)* mice during translational head-and-neck tumor progression. Moreover, using *in vitro* granulopoiesis model we have assessed maturation and properties of TANs in the context of tumor-derived factors and type I IFNs availability. Histological examination of murine bones was performed to analyze the migration of neutrophils and their progenitors from the bone marrow in response to cancer development.

Results: We could show that type I IFN deficiency results in the elevated neutrophil and their late precursor counts in hematopoietic organs during cancer progression. Moreover, our *in vitro* model demonstrates that the presence of tumor-derived factors stimulates accelerated development of neutrophils with activated pro-tumoral phenotype, while type I IFNs neutralize this phenomenon. Interestingly, according to our histology data both neutrophils and their precursors migrate from bone marrow more actively in WT than in *ifnar1-/-* mice. However, there are less WT neutrophils in the tumor, probably due to their accumulation in the spleen. The mechanisms of this phenomenon has to be still elucidated.

Conclusion: Our findings show that in cancer situation type I IFNs influence anti-tumoral properties of neutrophils already during their development in hematopoietic organs.

P69

Preclinical head and neck squamous cell carcinoma models for combined targeted therapy approaches

N. Schoenwaelder (Rostock/DE), B. Schneider (Rostock/DE), T. Freitag (Rostock/DE), M. Krause (Rostock/DE), D. Strueder (Rostock/DE), C. Grosse-Thie (Rostock/DE), C. Junghanss (Rostock/DE), C. Maletzki (Rostock/DE)

Introduction: Well-characterized, low-passage tumor models are the gold standard for preclinical research. Such models faithfully reflect the patients" individual molecular signature and treatment response.

Objectives: This study aimed at refinement of combined targeted approaches. Three individual HPV- head and neck squamous cell carcinoma (HNSCC) models were derived from locally advanced or metastatic patients. Comprehensive preclinical drug screening and response analysis was done in-depth.

Methods: Cell lines (HNSCC16 P1 M1, HNSCC46 P0 M2, HNSCC48 P0 M1) were treated with Cyclin-dependent kinase inhibitor (CDKI) abemaciclib (CDK4/6) +/- Cisplatin. Cyto-FISH was done for *CDKN2a*/*CDK4* to correlate CDKI response with molecular features. Functional analysis included immune-related phenotyping and expression status of drug-resistance genes.

Results: All cell lines were sensitive to Cisplatin. HNSCC48 P0 M1 was vulnerable to abemaciclib. NGS and Cyto-FISH identified *CDKN2a* loss (protein-truncating R58* mutation + monoallelic deletion). Besides, this cell line had chromosome 12 polysomy, accompanied by an increase of *CDK4*-specific copy numbers. In HNSCC16 P1 M1, but not HNSCC46 P0 M2, we likewise identified polysomy-associated *CDK4* gains. Although the former was not sensitive towards abemaciclib *per se*, these cells had CDKI-induced G1 arrest, an increased number of acidic organelles, and a swollen cell structure. Notably, intrinsic resistance was conquered by Cisplatin *via cMYC* and *IDO-1* downregulation. This combination significantly upregulated HLA-ABC on HNSCC16 P1 M1. Comparable effects were seen in HNSCC48 P0 M1. Such immunogenic effects were not visible in HNSCC46 P0 M2, the only highly resistant case in this study.

Conclusion: By performing functional and molecular analysis on patient-derived HNSCCs, we identified *CDK4* gains as "surrogate" marker for response to abemaciclib and describe a personalized approach to conquer intrinsic CDKI resistance.

P70
Functional characterization of neutrophil IFN signaling in cancer

I. Özel (Essen/DE), J. Jablonska (Essen/DE)

Background: In cancer, neutrophils play multifaceted roles that are regulated by environmental cues. Type I IFN signaling evidently regulates neutrophil functions in cancer, therefore, here; we aim to further study the mechanisms involved in this process.

Methods: We have created neutrophil-specific knockout mice for Stat3 (a negative downstream protein in IFN signaling) and assessed the neutrophil functions in such mice.

Results: Neutrophil-specific deletion of Stat3 increased the expression of CXCR2+ and CXCR4- on neutrophils in the bone marrow. However, the migration and phagocytosis ability of Stat3-deficient neutrophils did not significantly differ from WT neutrophils. Importantly, the ability of such neutrophils to form NETs and to promote angiogenesis were diminished.

Conclusions: *I*FN signaling plays a vital role on neutrophil functions in cancer. Here, we preliminarily show that disruption of Stat3 increases the pro-tumoral properties of neutrophils. Hence, fine-tuning of neutrophil signaling pathways hold great promise in cancer immunotherapy.

P71

Epigenetic priming of solid tumor cells enhances CAR T-cell cytotoxicity

C. Haist (Essen/DE, Düsseldorf/DE), C. König (Düsseldorf/DE), A. Bister (Essen/DE, Düsseldorf/DE), C. Wiek (Düsseldorf/DE), K. Scheckenbach (Düsseldorf/DE), G. Niegisch (Düsseldorf/DE), H. Hanenberg (Essen/DE), M. Hoffmann (Düsseldorf/DE), C. Grunewald (Düsseldorf/DE)

Introduction: Numerous preclinical studies have demonstrated that CARs against the target antigens EGFR and CD44v6 are universally applicable against a wide variety of solid tumors. However, the tremendous success of the clinically approved CAR T-cell therapies against B-cell malignancies could not be translated to the treatment of solid cancers yet, due to the limited efficacy of CAR T-cells in the tumor microenvironment and heterogenous antigen expression profiles.

Objectives: We investigated whether pretreatment of urothelial cancer cell lines (UCCs) with DNMTi decitabine (DEC) or HDACi romidepsin (ROM) will improve the lytic capacity of EGFR (Cetuximab) and CD44v6 (BIWA8) human CAR T-cells.

Materials and methods Four UCCs and a normal control cell line (HBLAK) were pretreated with 3 nM ROM (72 h) or 100 nM DEC (7 d), screened for their target antigen and T-cell ligand expression by flow cytometry and co-incubated overnight as target cells for 2nd generation CD44v6 and EGFR CARs lentivirally expressed in primary human T-cells in cytotoxic assays. Next generation RNA sequencing identified candidate genes whose expression was down-regulated by siRNAs.

Results: We demonstrated that exposure to DEC, but not ROM, enhanced the CAR T-cell cytotoxicity towards all UCCs, but not towards the uroepithelial HBLAK cells. Importantly, neither target antigen expression levels nor changes in T-cell adhesion molecules, but regulators of cell survival and apoptosis were identified as differentially modulated by the two treatment regimens. Knockdown experiments with siRNA finally confirmed BID and BCL2L1 as two key factors regulating sensitivity of DEC-treated UCCs towards cytotoxic lysis by CAR T-cells.

Conclusion: We showed that low-dose epigenetic modulation of tumor cells with DEC can overcome barriers that limit CAR T-cell effectiveness. Ultimately, this combined therapy of epigenetic drugs and immunotherapy should be evaluated for the treatment of solid tumors in general.

P72

Mapping the tumor microenvironment of squamous cell carcinomas of the head an neck by multiplex immunohistochemistry

C. Netzer (Göttingen/DE), A. Weigert (Frankfurt a. M./DE), D. Beutner (Göttingen/DE)

Interactions between tumor cells and the tumor microenvironment play a key role in tumor development and metastasis. Both the composition of the tumor microenvironment and the localization of the cells in the tumor have an impact on its further development. Immunohistochemistry has been the method of choice over years for examining solid tumors because sample preparation is straightforward and paraffin tissue in particular can be stored for long periods of time. Multiplex immunohistochemistry represents a relatively new approach that allows simultaneous quantification of multiple antigens. A great advantage over flow cytometry or next generation sequencing is that it allows statements to be made about the tumor morphology and spatial cell/cell relationships. One of the major logistical hurdles here is the large amount of data generated during such analyses and the heterogeneity between samples. Here, we share different on R programming language based workflows for analyzing the tumor microenvironment in squamous cell carcinoma of the head and neck region using multiplex immunohistochemistry, which our group has developed over the past few years. These include workflows to determine the coexpression of proteins within a cell, the cell density, as well as of cell neighborhoods. This demonstrates that multiplex immunohistochemistry is a highly effectively tool to identify and quantify cell populations, establish proximity relationships between the cells and compare samples with a high degree of heterogeneity.

P73

Are there beyond PD-L1-expression predictors of outcome for patients with recurrent or metastatic head and neck cancer?

C. Plettenberg (Düsseldorf/DE), L. Althaus (Düsseldorf/DE), J. Hausmann (Düsseldorf/DE), B. Tamaskovics (Düsseldorf/DE)

Purpose: PD1 antibodies (PD1AK) have been shown to be effective in relapsed / metastatic head and neck cancer (rmHNC), albeit with a relatively low response rate. Predictive factors, besides TPS and CPS, would be desirable. For this reason, we examined the number and distribution of haemological factors (HF) and examination data of patients on PD1AK therapy in relation to the outcome.

Material and methods: The following pretherapeutic findings were retrospectively collected from 81 patients with rmHNC and PD1AK therapy (2017-2021) and their outcome was analyzed: complete blood count (CBC), baseline levels of albumin, haemoglobin, lactate dehydrogenase (LDH) and C-reactive-protein (CRP), CPS, TPS, alcohol consume and smoking habits, body mass index (BMI), Zubrod- (ECOG) and Karnofsky performance scores (KPS) were collected retrospectively. We calculated Neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR) from CBC and modified Glasgow Prognostic Score (mGPS) from serum CRP and albumin levels. Overall survival was estimated with the Kaplan-Meier method, hazard ratios with uni-/multivariate Cox regression models (R version 4.1.1).

Results: The median follow-up time was 18.2 months, the median OS 7.8 months (95% confidence interval [CI]: 5.2-13.8 months). ECOG and KPS were good predictors of the outcome. PD-L1-expression showed a negative impact. Smoking history (>10 pack years) and CPS were independent prognostic factor (HR: 3.3/2,9; CI: 1.7-6.5/1.1-7.3; $p < .001/=0.27$). All six investigated inflammatory biomarkers showed a negative impact on the outcome: low baseline haemoglobin, LDH-elevation, elevated NLR, elevated MLR, elevated PLR and mGPS > 0.

Conclusion: Present results demonstrate that besides PD-L1-expression, baseline patient performance and smoking history the investigated hematologic and serum biomarkers of inflammation are prognostic factors for patients with rmHNC treated with PD-1-inhibitors

P74

Intraoperative hyperspectral imaging for decision making in head and neck cancer

S. Wiegand (Leipzig/DE), A. Dietz (Leipzig/DE), G. Wichmann (Leipzig/DE), M. Maktabi (Leipzig/DE), C. Chalopin (Leipzig/DE)

Introduction: Novel intraoperative imaging systems may have a critical impact on intraoperative decision-making in head and neck cancer. Hyperspectral imaging (HSI) is an emerging new technology which incorporates traditional imaging and spectroscopy together to obtain both spatial and spectral information from tissues simultaneously in a non-invasive manner. This system provides colored pictures indicating the oxygen saturation (StO2), tissue perfusion (NIR-PI), organ hemoglobin index (OHI) and tissue water index. The aim was to evaluate the use of intraoperative HSI in patients with head and neck cancer.

Methods: Intraoperative HS images were recorded as part of a pilot study during tumor surgery as well as reconstructive surgery with free and pedicled flaps in head and neck cancer. For the acquisition of HSI data, the TIVITA® Tissue System (Diaspective Vision GmbH) was used.

Results: Intraoperative HSI was possible in all patients and did not prolong the regular operative procedure due to its quick applicability. First results demonstrate that HSI helps to identify critical anatomical structures and to distinguish tumor from healthy tissue. Moreover, it can be used to monitor the circulation of free and pedicled flaps.

Conclusion: HSI is suitable for contact-free, non-invasive, and intraoperative evaluation of tissue parameters. It can be used to guide surgery, to examine tumor resection margins in ex vivo surgical specimen in the operating room, and to monitor tissue oxygenation of microvascular free and pedicled flaps in reconstructive surgery.

P75

How to improve outcome in head and neck cancer – take a look at neutrophils

B. Antuamwine (Essen/DE), Y. Si (Essen/DE), B. Wang (Essen/DE), M. Elkabets (Beer-Sheva/IL), K. S. Lang (Essen/DE), A. Squire (Essen/DE), S. Brandau (Essen/DE)

Background: A high frequency of tumor-associated neutrophils (TAN) and T cell suppression are associated with poor prognosis of HNC patients and with immunotherapy failure. Here, we aim to understand the functional dynamics of TAN-T cell interplay in HNC, in order to identify targets for therapy optimization.

Methods: We analysed the functional state and spatial arrangement of TAN and T cells in human HNC tissue by novel multi-modal imaging technologies. We established two HNC models in immunocompetent C57BL/6 mice and investigated the functional dynamics of neutrophils and T cells during tumor progression by flow cytometry and immunofluorescence.

Results: Novel imaging techniques allowed 3-D analysis of intratumoral immune cells. We identified an intratumoral TAN subset that inhibits T cell activity in human HNC tissue. Patients with high frequencies of suppressive TAN showed poor outcome. Targeted depletion of TAN in a murine SCC tongue model enhanced T cell frequencies in the tumor microenvironment.

Conclusion: Our study provides a mechanistic explanation for the high clinical relevance of TAN frequencies in HNC and suggests new strategies to optimize immunologic therapies.

P76

Immunotherapy targeting PD-1 in advanced head and neck squamous cell carcinoma

B. Vamosi (New Franklin/US), D. Aziz (Cincinnati, OH/US), S. Bhati (Cincinnati, OH/US), E. Manders (Cincinnati, OH/US), M. Bhandari (Cincinnati, OH/US)

Educational objective: At the conclusion of this presentation, the participants should be able to discuss the treatment options for patients with Squamous Cell Carcinoma (SCC) affecting the head and neck.

Objective: To study the effects of Libtayo, an immunotherapy targeting PD-1, on Squamous Cell Carcinoma (SCC) in a non-optimal surgical candidate.

Study Design: Case report.

Methods: An 88-year-old male patient with biopsy proven SCC of the right ear was treated with immunotherapy, Libtayo, for 10 doses of 350mg every three weeks. Patient's chart was further reviewed for presentation, workup, diagnosis, and treatment.

Results: Patient was given the surgical option of a total auriculectomy and lateral temporal bone resection. The patient along with his ENT and oncology teams decided to proceed with immunotherapy.

Pre-treatment CT showed the lesion was 4.3 cm x 4 cm x 2.7 cm and extended into the right auditory canal. Patient had significant hearing loss related to the lesion.

Post-treatment CT showed residual soft tissue thickening with no measurable lesion. Biopsy showed granulation tissue with no signs of SCC. Audiogram was declined, but there was a significant subjective improvement in hearing at the end of treatment.

Conclusions: SCC treatment generally includes excision with low recurrence rates with excision alone. SCC affecting the head and neck is more commonly a high-risk lesion that requires larger margins and has higher recurrence rates. Currently non-surgical options are limited and have higher recurrence rates. In this patient, Libtayo has shown promising results including drastic reduction in lesion size and almost complete reversal of hearing loss. While long-term mortality and morbidity for this treatment cannot be determined at this time, Libtayo could provide an additional or alternative treatment option for patients.

P77

Small extracellular vesicles from head and neck squamous cell carcinoma cells carry a proteomic signature for tumor hypoxia

A. Gluszko (Warsaw/PL), M. Szczepański (Warsaw/PL), T. L. Whiteside (Pittsburgh, PA/US), T. Reichert (Regensburg/DE), J. Siewiera (Warsaw/PL), N. Ludwig (Regensburg/DE)

Introduction: Tissue hypoxia is a hallmark of head and neck squamous cell carcinoma (HNSCC) and is considered to drive tumor progression and resistance to anti-cancer therapies. Small extracellular vesicles (sEVs) are a potential resource for monitoring tissue hypoxia in HNSCC or even anti-angiogenic or vessel normalization therapies.

Objectives: The aim of this study was to characterize the influence of hypoxic environments on the release and proteomic cargo composition of sEVs.

Material and methods: HNSCC cells (FaDu, PCI-30, SCC-25) and HaCaT keratinocytes were cultured in 21, 10, 5, 1% O2. sEVs were isolated from supernatants using size exclusion chromatography (SEC) and characterized by nanoparticle tracking analysis, electron microscopy, immunoblotting, and high-resolution mass spectrometry.

Results: Isolated sEVs ranged in size from 125-135 nm and contained CD63 and CD9 but not Grp94. sEVs reflected the hypoxic profile of HNSCC parent cells: about 15% of the total detected proteins were unique for hypoxic cells. Hypoxic sEVs expressed a common signature of seven hypoxia-related proteins (KT33B, DYSF, STON2, MLX, LIPA3, NEK5, P12L1) and were enriched in pro-angiogenic proteins. Adaptation of HNSCC cells to hypoxia was associated with increased release of sEVs, which were enriched in a unique protein profile.

Conclusion: Protein profiles of sEVs reflected the degree of tumor hypoxia and could serve as a potential sEV-based biomarkers for hypoxic conditions.

P78

Human papilloma virus-16 and tumor stage – Is there a correlation?

M. Gomaa (Minia/EG), U. Abdel Nabi (Minia/EG), K. Elgendy (Minia/EG)

Objective: Many patients with Laryngeal squamous cell carcinoma (LSCC) do not have any of the traditional risk factors associated with head and neck squamous cell cancers. Epidemiological and molecular studies have identified human papillomavirus (HPV) as a causative agent.

The aim of the study is to find the relation between HPV-16 infected LSCC and T and N stage of the tumor.

Study Design: Prospective, cross section

Setting: Tertiary university hospital

Patients and methods: The current study was conducted on 47 cases suffered from LSCC, all patients subjected to clinical, radiological and endoscopic assessment of the tumor. Biopsy was taken from each patient and stained by H&E to confirm the clinical diagnosis and also Immunohistchemical staining was done to evaluate infected tumors by HPV-16. SPSS program version 16 was used to assess the correlation between HPV-16 affected tumors and T and N stage of the LSCC.

Results: The study sample pointed out that the majority of patients were in T2 and N0 stage. The current study showed that most of the patients infected with HPV-16 presented in T2 stage (six out of nine patients 66.7%), while the majority HPV-16 negative patients also presented in the same stage (36 patients 94.7%). There is no difference between HPV-16 positive and negative patients in N stage as the majority of both groups presented in N0, 66.7% in positive cases compared to 76.3% in negative cases. Our results reveal that 22.2% of HPV positive cases were in stage T3 and T4 compared to 0% in the HPV negative cases in the same stages. The second common presenting N stage of HPV-16 positive cases was N2, while in HPV negative patients was stage N1.

Conclusion: Our results showed that there were no correlation between T nor N stage of HPV-16 infected LSCC, but indeed not statistically significant we found that HPV positive cases tend to be presented more in advanced T and N stage compared to HPV negative cases.

P79
HPV and laryngeal cancer

M. Gomaa (Minia/EG), U. Abdel Nabi (Minia/EG), K. Elgendy (Minia/EG), O. Abd Elaal (Minia/EG)

Introduction:- Laryngeal cancer is the most common type of cancer in the head and neck. Human papilloma virus (HPV) represents a group of more than 150 related viruses. Infection with certain types of HPV can also cause some types of cancer. HPV now causes most OroPharyngeal Cancer, Common sites base of the tongue and tonsil. HPV as a cause of Cancer larynx is still unclear.

Overview of the course:- The course will reveal all risk factors of laryngeal carcinoma, role of HPV in oropharyngeal cancer, global prevalence of HPV in cancer larynx, epidemiology of HPV in laryngeal cancer, clinical implication and prognosis of HPV positive cases with cancer larynx, comparison between HPV positive cases with laryngeal and oropharyngeal cancer.

Learning outcome:- at the end of the course attendees will lean, risk factors of cancer larynx including HPV, epidemiology of HPV in laryngeal cancer, pathophysiology of laryngeal carcinoma when infected by HPV, comparison between Oropharyngeal and laryngeal carcinoma that infected by HPV. Prognosis of HPV infected laryngeal carcinoma and biological difference between HPV positive and negative laryngeal carcinoma.

P80

Preliminary insights into the impact of primary radiochemotherapy on the salivary microbiome in head and neck squamous cell carcinoma

A. Wolf (Graz/AT), C. Kumpitsch (Graz/AT), C. Moissl-Eichinger (Graz/AT), A. Perras (Regensburg/DE), K. Koskinen (Graz/AT), P. V. Tomazic (Graz/AT), J. Pock (Graz/AT), P. Pondorfer-Schaefer (Graz/AT), D. Thurnher (Graz/AT)

Squamous cell carcinoma is the most common type of throat cancer. Treatment options comprise surgery, radiotherapy, and/or chemo(immuno)therapy. The salivary microbiome is shaped by the disease, and likely by the treatment, resulting in side effects caused by chemoradiation that severely impair patients' well-being. High-throughput amplicon sequencing of the 16S rRNA gene provides an opportunity to investigate changes in the salivary microbiome in health and disease. In this preliminary study, we investigated alterations in the bacterial, fungal, and archaeal components of the salivary microbiome between healthy subjects and patients with head and neck squamous cell carcinoma before and close to the end point of chemoradiation ("after"). We enrolled 31 patients and 11 healthy controls, with 11 patients providing samples both before and after chemoradiation. Analysis revealed an effect on the bacterial and fungal microbiome, with a partial antagonistic reaction but no effects on the archaeal microbial community. Specifically, we observed an individual increase in Candida signatures following chemoradiation, whereas the overall diversity of the microbial and fungal signatures decreased significantly after therapy. Thus, our study indicates that the patient microbiome reacts individually to chemoradiation but has potential for future optimization of disease diagnostics and personalized treatments.

P81

Evaluation of the impact of 8th version of tumor classification for p16-positive and p16-negative oropharyngeal cancers in daily clinical routine

P. Pondorfer-Schaefer (Graz/AT), A. Strasser (Graz/AT), A. Wolf (Graz/AT), S. Vasicek (Graz/AT), P. Kiss (Graz/AT), T. Weiland (Graz/AT), L. Brcic (Graz/AT), K. Walla (Graz/AT), D. Thurnher (Graz/AT)

Background: The 8th edition of UICC TNM classification (TNM8) gives a precise prognosis for patients with OPSCC (oropharyngeal squamous cell carcinoma). Tumor stage II&III remain indistinguishable, further, recommendations for therapy changes based on TNM8 are missing, resulting in different decisions for patients in head and neck tumor boards (HNTB) worldwide. In the era of TNM update, we evaluated therapy courses, patient outcomes, and tumor staging in our comprehensive cancer center (CCC) HNTB.

Methods: Medical records of 178 patients with OPSCC diagnosed between 2015-2018 were analyzed retrospectively. All patients were re-staged by two independent research associates according to 7th and 8th TNM editions.

Results: Before TNM8 implementation, in 20% and after TNM8 implementation in 36.5% staging revision was needed, usually patients were staged too low. Patients with early p16-positive OPSCC were treated with surgery (before TNM8:10.4% vs after TNM8:0%), surgery, and chemoradiotherapy (before TNM8:37,5% vs TNM8:30%) or primary chemoradiotherapy (before TNM8:52% vs after TNM8:70%; ns). Patients with advanced p16-positive OPSCC were mainly treated with primary chemoradiotherapy (before TNM8:90,9% vs after TNM8:100%). Complete remission rate at first re-staging was higher after implementation of TNM8 in early OPSCC. Undergoing surgery predicts higher chances for complete remission regardless of staging.

Conclusion: 8th edition of TNM enables a better prognosis for p16pos OPSCC. Changes in TNM classification do not affect therapy courses significantly, although a shift towards primary chemo-radiotherapy was observed. This study has proven high quality in CCC HNTB, nevertheless TNM8 may affect decisions unwittingly, monitoring institutional therapy trends is an important proof of quality tool.

P82

Novel insights into RNA methylation in head and neck squamous cell carcinoma

K. Romanowska (Poznán/PL), W. Golusiński (Poznán/PL)

Background: RNA methylation at nitrogen sixth of adenosine (m6A, N6-methyladenosine) is the most abundant RNA modification which plays a crucial role in all RNA metabolic aspects. Recently, m6A modification has been assigned to mediate the biological processes of cancer cells, but their significance in HNSCC development is still poorly described.

Objectives and methods: Thus, the main aim of our study was to globally quantify m6A modification by mass spectrometry approach and mRNA expression level of selected m6A RNA methyltransferase (METTL3), demethylase (FTO), and m6A readers (YTHDF2, YTHDC2) in 45 HNSCC patients, 4 cancerous cell lines (FaDu, Detroit 562, A-253 and SCC-15) and primary gingival keratinocytes cell line (PCS-200-014) using qPCR.

Result: We have not observed differences in the global amount of m6A modification and mRNA level of selected genes between cancerous and paired-matched histopathologically unchanged tissues from HNSCC patients. However, we have found a positive correlation of m6A modification with selected m6A RNA methyltransferase, demethylase and binding proteins on total RNA and characterized the transcript level of those genes in HNSCC cell lines.

Conclusion: In brief, our results indicate the positive correlation of m6A modification with selected m6A RNA methylation machinery genes in HNSCC. Moreover, the lack of global m6A differences between cancerous and histopathologically unchanged tissues suggests that alterations of m6A at individual RNA may specifically influence HNSCC tumorigenesis.

P83
Mesenchymal / epithelial switch in head and neck cancer

J. Dudas (Innsbruck/AT), J. Ingruber (Innsbruck/AT), M. D. C. Greier (Innsbruck/AT), T. Steinbichler (Innsbruck/AT), R. Rohregger (Innsbruck/AT), H. Riechelmann (Innsbruck/AT)

Question: Therapy resistance of head and neck squamous cell carcinoma (HNSCC) is close related with the epithelial/mesenchymal plasticity of the HNSCC tumor cells.

Methods: Collection of HNSCC tumor tissue, gene expression analysis using real-time PCR, cell culture models, treatments with growth factors, ectopic overexpression, proliferation assays, statistical analysis, immunohistochemistry and quantitative image cytometry.

Results: Epithelial to mesenchymal transition (EMT) is related with poor response to radiochemotherapy (RT/CRT), invasion, and an increased cell migration potential. In head and neck squamous cell carcinoma tissue the detection of EMT was based on complex multiple immunofluorescence, but in 2020 we described a surrogate marker Slug, whose overexpression efficiently characterized EMT. EMT was regulated by intrinsic factors as p53 mutational status, human papilloma virus background, by environmental conditions as transforming-growth-factor-beta-1 (TGF-beta-1) or interleukin-6, as well as by cellular stress as mitochondrial dysfunction and lack of growth factors and nutrients. Transcription factors engaged with either the mesenchymal phenotype as Slug, or with the epithelial one as Krüppel-like-factor-4 (KLF4) were able to regulate the replacement of epithelial and mesenchymal phenotype in HNSCC tumor cells. Using SCC-25 cells, we achieved a reproducible EMT – mesenchymal-to-epithelial (MET) model by treatments with TGF-beta1 and subsequent KLF4-overexpression. The mesenchymal cells moved actively, were invasive but did not grow, whereas after re-activation of the epithelial phenotype the cells started to proliferate and divided.

Conclusions: Clinically relevant model for the EMT/MET switch is required for tailored therapy for disseminating tumor cells and for outgrowth of relapse and metastatic nests.

P84

High content image analysis and machine learning applied to H&E stains identifies potential prognostic factors in HPV+ oropharyngeal squamous cell carcinoma

J. Hue (London/GB), Z. Valinciute (London/GB), S. Thavaraj (London/GB), L. Veschini (London/GB)

Introduction: Patients with Human Papillomavirus positive oropharyngeal Squamous Cell Carcinoma(HPV+opSCC) have better prognosis than HPV- counterparts, raising the possibility of treatment de-escalation in the former. However, about 20% of HPV+opSCC patients demonstrate poor prognosis, contraindicating de-escalation regimes in this subgroup. Hence, it is important to identify patients who are unlikely to benefit from treatment de-escalation.

Objectives: We aimed to develop an automated workflow for quantitative image analysis of H&E stained sections of HPV+opSCCs. Using this workflow, we measured tumour-infiltrating lymphocytes(TILs), taking into account their spatial relationship with tumour cells and stroma, stromal plasma cells, and tumour nuclei features.

Methods: 58 HPV+opSCC patients were retrospectively identified, of whom 30 were disease-free (favourable prognosis) and 28 demonstrated recurrence or had died of disease within 5 years (unfavourable prognosis). Ten representative H&E-stained photomicrographs at 100x magnification were acquired from each specimen. Images were analysed with a dedicated open-source workflow using machine learning(ML) algorithms for image segmentation and object classification, followed by multivariable statistical analysis.

Results: 18 of 21 variables were statistically significant (Student"s T-test). A multivariable Hotelling"s T2 test was also performed with all variables(P<0.0001). A multivariable logistic regression model revealed significant differences between favourable and unfavourable outcomes in TIL index(P=0.021), plasma cells(P=0.042), tumour cell area(P=0.005), compactness(P=0.020), neighbour distance(P=0.018) and texture(P=0.0002). The data was then fitted to a quadratic discriminant analysis. This preliminary model had an accuracy of 100.0% to predict unfavourable and 96.7% for favourable prognosis on our cohort.

Conclusion: Our open-source H&E analysis workflow and model can prognosticate HPV+opSCCs with promising accuracy. Our work supports the use of ML in digital pathology to exploit clinically relevant features in routine diagnostic pathology without additional biomarkers.

P85

Identification of a predictive marker signature for diagnosing HNSCC based on platelet RNAseq

C. Brunner (Ulm/DE), L. T. Huber (Ulm/DE), J. M. Kraus (Ulm/DE), J. Esic (Ulm/DE), A. Wanli (Ulm/DE), M. Groth (Jena/DE), S. Laban (Ulm/DE), B. Wollenberg (Munich/DE), H. A. Kestler (Ulm/DE), T. K. Hoffmann (Ulm/DE)

Introduction: Liquid biopsy offers a way identifying cancer by examination of body fluids. The present study deals with the analyses of "tumor-educated platelets" (TEP), a recently discovered novel option of liquid biopsy. Previous research identified a tumor cell – platelet interaction in different tumor entities, resulting in a transfer of tumor derived RNA into platelets, named further TEP.

Material and methods: Sequencing analysis of RNA derived from platelets of tumor patients and healthy donors was performed. Additionally, RNA from the corresponding tumor was sequenced. Bioinformatic tools were applied. Subsequently, quantitative RT-PCR was used for verification of differentially existing mRNA in platelets from tumor patients versus healthy donors in a second cohort.

Results: RNAseq data revealed 426 significantly differentially existing RNA. Among them, we identified RNA coding for 49 genes characteristically expressed in epithelial cells. Additionally, in tumor patient"s platelets we observed RNA coding for genes involved in tumor progression by contributing to proliferation, metastasis or angiogenesis. We identified 5 differentially existing mRNA as potentially liquid biopsy biomarkers in TEP.

Conclusion: Based on these promising results of this pilot study a prospective study including a larger cohort should be initiated in order to verify the here proposed predictive marker signature allowing the identification of HNSCC based on platelet RNAseq.

P86
Clinical case – primary trachea cancer

S. Yashin (Samara/RU)

Patient S., 57 y. o., was admitted in serious condition with complaints of severe shortness of breath, subjective feeling of shortness of breath, subfebrile fever. During the examination, central lung cancer was suspected, computed tomography was performed: a tumor was visualized in the region of the lower third of the trachea with a transition to the main bronchi, metastases to the lymph nodes. Despite the treatment, the patient's condition remained serious. On the third day of hospitalization, the patient was diagnosed with biological death.

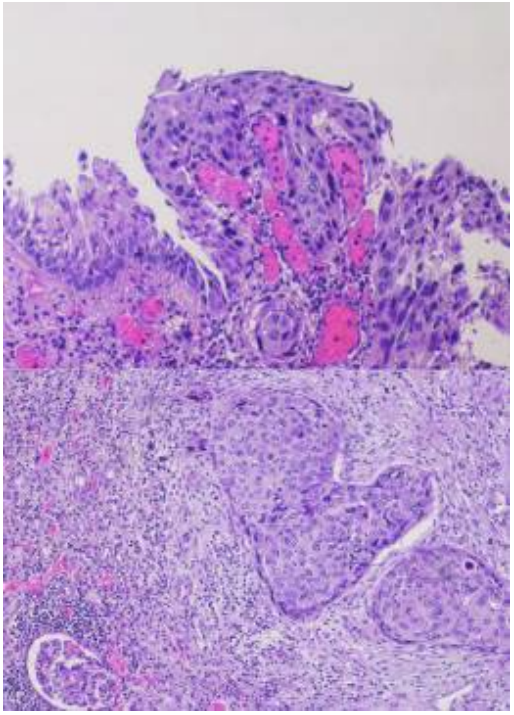
Pathological examination: In the area of the trachea bifurcation with the transition to the main and lobar bronchi, the wall of the trachea and bronchi is sharply thickened, represented by a white dense tissue, spreading to the lung tissue in the area of the roots. In the area of the upper pole of the left kidney, there is a section of dense white tissue with indistinct boundaries, 1 cm in diameter.

Histological examination: Trachea and main bronchi. Microscopic examination shows moderate blood filling, proliferation of tumor tissue with the formation of "pearls", with foci of necrosis and leukocyte infiltration in tumor complexes, pronounced fibrosis and lymphoid infiltration in the stroma. The epithelium of the trachea is replaced by tumor tissue in many areas.

Lungs: On histological examination, growth of tumor tissue (squamous cell keratinizing cancer) is observed in the basal regions, with severe fibrosis and moderate inflammatory infiltration.

Microscopic examination of the kidneys reveals an area of tumor tissue, pronounced fibrosis and lymphoid infiltration, mainly around the tumor tissue. Final postmortem diagnosis: cancer of the lower third of the trachea with spread to the right and left main bronchi (squamous cell carcinoma). Metastases to the lungs and left kidney (T4N1M1). The pathogenesis of the development of primary tracheal cancer is still insufficiently understood and is of great interest for study.

Figure



EHNS
European Head & Neck Society