Methods: After pathological re-evaluation, we analysed 360 cancer genes in tumours and matched blood from 172 HG GEP-NEN patients; 147 neuroendocrine carcinomas (NEC) and 25 neuroendocrine tumours (NET G3).

Results: For NEC, frequently mutated genes were *TP53* (65%), *APC* (28%), *KRAS* (22%) and *BRAF* (20%). *RB1* was only mutated in 14%, but CNAs affecting *RB1* were seen in 48%. Other frequent losses were *ARID1A* (48%), *ESR1* (41%) and *ATM* (45%). Frequent amplifications were found in *MYC* (50%) and *KDM5A* (46%). While these molecular features had limited similarities with SCLC, we found potentially targetable mutations in 72% of the NEC samples. Mutations and CNA varied according to primary tumour site with *BRAF* mutations mainly seen in colon (49%), and *FBXW7* mutations mainly seen in rectal cancers (25%). 9/147 (6%) NEC were MSI. Alterations affecting *TP53* and *RB1* signalling were associated with improved prognosis. NET G3 had frequent mutations in *ATRX* (16%), *MEN1*, *MYO5B*, *SF3B1*, *SMAD2* and *TP53* (each 12%).

Conclusions: We performed a comprehensive assessment of the molecular tumour alterations in a large series of gastroenteropancreatic high-grade neuroendocrine neoplasms. We found few RB1 mutations and a marked difference in the molecular profile compared to prior results in SCLC and LCLC, challenging the use of SCLC as a paradigm for GEP-NEC. We found a quite similar profile comparing large-cell and small-cell GEP-NEC, but a profile variation according to primary tumour site and suggest a possible molecular strategy to separate NEC from NET G3. Our study shows a very high fraction of GEP-NEC with targetable mutations, pointing to novel important therapeutic strategies.

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1101MO Development of CAR T-cells for future treatment of NETs

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Background: Neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs). We investigated the antitumor activity of chimeric antigen receptor (CAR) T cells directed against SSTRs.

Methods: A second-generation CAR-like construct containing two molecules of octreotide in the extracellular moiety and CD28 as costimulatory module was cloned in a pMSGV1-28Z retroviral vector and then transduced in human T cells. Luciferase$^+$ (Luc$^+$) BON1, CM, QGP1, CNDT2.5 and H727 NET cell lines were screened for membrane $SSTR_{2/5}$ expression by Western blot (WB) and flow cytometry. Co-culture experiments were performed at effector:target (E:T) ratios ranging from 50:1 to 1:50 for up to 72 hrs. Tumor cell cytotoxicity was assessed by bioluminescence imaging. The release of IFN-γ and IL-2 by activated CAR T cells was investigated by ELISA. NSG female mice (n=11/group) were subcutaneously injected with $2x10^6$ Luc$^+$ BON1 or CM cells, and were then intravenously treated either with $7x10^6$ anti-SSTR CAR T cells, or untransduced (UT) T cells. Excised tumors were subjected to PCR to assess the infiltration of CAR T cells. Potential on-target off-tumor toxicities of anti-SSTR CAR T cells were investigated by pathological analysis of mouse brain and pancreas.

Results: All NET cell lines expressed $SSTR_{2/5,}$ although at variable levels. Following WB confirmation of the CAR expression by transduced lymphocytes, anti-SSTR CAR T cells were co-incubated with target cells. Tumor cell death was induced in approximately 40% ($\pm$8%) of CM and BON1 cells at E:T ratio of 1:1. The tumoricidal effect of CAR T cells was time-dependent and peaked at 72 hrs. Compared with UT T cells, CAR T cells secreted significantly higher levels ($p<0.01$) of IFN-γ and IL-2 after co-incubation with NET cells. Anti-SSTR CAR T cells effectively infiltrated tumors and significantly reduced the growth of subcutaneous CM ($p=0.01$) and BON1 xenografts ($p=0.02$) in mice by *in vivo* bioluminescence imaging. No pathological alterations were seen in the brain and pancreas of mice treated with CAR T cells.

Conclusions: Anti-SSTR CAR T cells exert antitumor activity against SSTR$^+$ NET cell lines, both in vitro and in vivo.

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1102P The phase III NETTER-1 study of 177Lu-DOTATATE in patients with midgut neuroendocrine tumours: Further survival analyses

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Background: In the prespecified final analysis of overall survival (OS) in the NETTER-1 study, with median follow-up of more than 6.3 years, ^{177}Lu-DOTATATE prolonged median OS by 11.7 months versus high-dose octreotide in patients with advanced, progressive, well-differentiated midgut neuroendocrine tumours, although OS difference did not reach statistical significance (HR, 0.84 [95% CI: 0.60, 1.17]; $p = 0.30$). This analysis used the proportional-hazards assumption.

Methods: In this international open-label trial, 231 patients were randomized 1:1 to receive four cycles of ^{177}Lu-DOTATATE 200 mCi every 8 weeks plus long-acting octreotide 30 mg (^{177}Lu-DOTATATE arm), or long-acting octreotide 60 mg every 4 weeks (control arm). OS was evaluated in subgroups of age, Karnofsky performance status and baseline tumour burden (OctreoScan). The proportional-hazards assumption for the Cox regression analysis of OS was examined using Schoenfeld residuals. Analysis of restricted mean survival time (RMST; area under the survival curve up to a specific time point) at 2, 3, 4 and 5 years was specified before final data cut-off to estimate treatment effect in the presence of non-proportional hazards.

Results: OS was consistent across most prespecified subgroups. During long-term follow-up, more than 60% of patients in each arm received further anti-cancer therapies, including 'cross-over' to peptide receptor radionuclide therapy (PRRT) in 36% of patients in the control arm. The proportional-hazards assumption for the Cox analysis of OS was not met ($p = 0.034$). RMST was numerically longer in the ^{177}Lu-DOTATATE arm versus control at all timepoints (table).

Table: 1102P Restricted mean survival time analysis at 2, 3, 4 and 5 years

Time since randomization, years		^{177}Lu-DOTATATE N = 117	Control N = 114
2	Deaths, n (%)	26 (22.2)	39 (34.2)
	RMST, months (95% CI)	21.2 (20.2, 22.3)	19.3 (18.0, 20.7)
	Difference, months (95% CI)	**1.9 (0.1, 3.6)**	
3	Deaths, n (%)	41 (35.0)	51 (44.7)
	RMST, months (95% CI)	29.7 (27.7, 31.6)	26.0 (23.7, 28.3)
	Difference, months (95% CI)	**3.7 (0.7, 6.7)**	
4	Deaths, n (%)	53 (45.3)	58 (50.9)
	RMST, months (95% CI)	36.2 (33.4, 39.0)	31.5 (28.3, 34.8)
	Difference, months (95% CI)	**4.6 (0.3, 8.9)**	
5	Deaths, n (%)	65 (55.6)	63 (55.3)
	RMST, months (95% CI)	41.2 (37.6, 44.9)	36.1 (31.9, 40.4)
	Difference, months (95% CI)	**5.1 (−0.5, 10.7)**	

Conclusions: Prespecified final analysis of OS was likely confounded by multiple factors, including cross-over to PRRT and a high 5-year survival rate, confirmed by non-proportional extensions of RMST over time. Nevertheless, ^{177}Lu-DOTATATE prolonged median OS by 11.7 months.

Clinical trial identification: Protocol number: AAA-III-01. Release date: 5 June 2014. EudraCT 2011-005049-11.