A prospective study of cancer survival in patients with HuD-antibody associated paraneoplastic neurological disorders

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Supplementary Methods

Antibody analysis

Initial diagnostic samples tested in Oxford were screened for the presence of antineuronal antibodies on rat cerebellum and brain stem substrate following incubation at 1:100 for 30 minutes. After removal of unbound antibodies and subsequent incubation with fluorescein conjugated anti-human IgG (1:100), staining patterns were analysed on a fluorescence microscope (Olympus BX41) by two observers. Results indicating a positive anti-neuronal staining pattern underwent confirmatory testing using commercial immunoblots (Ravo Diagnostika, Freiburg, Germany). For further verification, 36 samples were tested additionally using western blotting to recombinant HuD at serum dilutions between 1:1000 to 1:4000, as previously described (Parsy et al, 2007). SOX2 and HuD antibodies were detected using a semi-automated ELISA (Chapman et al, 2011).

Chapman CJ, Thorpe A, Murray A, Kite T, Woodard K, Maddison P, et al. Autoantibodies in small cell lung cancer provide an opportunity for early detection Clinical Cancer Research 2011; 17: 1474-80.

Parsy CB, Chapman CJ, Barnes AC, Robertson JF, Murray A. Two-step method to isolate target recombinant protein from co-purified bacterial contaminant SlyD after immobilised metal affinity chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 853: 314-9.

Supplementary Table 1. Summary of demographics, clinical syndromes, autoantibodies and survival data for each group and subgroup.

	Hu-Ab PND all	Hu-Ab PND SCLC	Hu-Ab PND other tumours*	Hu-Ab PND no tumour detected	SCLC	SCLC Hu-Ab Negative
Number in subgroups (% cohort)	103 (100%)	64/103 (62%)	25/103 (24%)	14/103 (14%)	245 (100%)	223 (91%)
Median age (range) at PND diagnosis (years)	67 (3 – 89)	67 (40-79)	64 (10 – 89)	68 (3 – 83)	N/A	N/A
Median age at cancer diagnosis	66.3 (9.1 – 89.3)	66.4 (40.1 – 79.2)	65.8 (9.1 – 89.3)	N/A	66 (33-87)	66 (33-87)
Females (%)	62 (60.2%)	39 (60.9%)	15 (60%)	8 (57.1%)	121 (49.4%)	106 (47.5%)
Classical PNDs (pure neurological presentation)	24 SSN, 9 PCD, 10 LE, 1 Autonomic	18 SSN, 4 PCD, 5 LE	4 SSN, 4 PCD, 3 LE	2 SSN, 1 PCD, 2 LE	None	None
Mixed presentation predominantly central (LE/BE/ataxia)	27 (26.2%)	19/64 (29.7%)	6/25 (24%)	2/14 (14.3%)	None	None
Mixed presentation predominantly peripheral (neuropathy, LEMS)	32 (31.1%)	18/64 (28%)	8/25 (32%)	6/14 (43%)	None	None
HuD-Abs, mean titre (OD) ± SD	1.86 ± 0.38	1.92 ± 0.28	1.86 ± 0.51	1.62 ± 0.44	1.66 ± 0.43 (n=22)	None
SOX-Abs, (% of subgroup)	37/97 (38%)	31/60 (52%)	5/23 (22%)	1/14 (7%)	71 (29%)	57 (26%)
Time (mo.) from PND to death: median (range; no. censored)	14 (0.25 – 126; 27)	14 (0.75 – 111; 15)	10.8 (0.25 – 124; 6)	28 (4 – 126; 6)	N/A	N/A
Time (mo.) from cancer to death: median (range; no. censored)	11 (0 - 108; 21)	11.5 (0 - 108; 15)	7.0 (1-100; 6)	N/A	9.75 (0.25 - 119; 6)	9.25 (0.25 - 119; 6)

BE = brainstem encephalitis; LE = limbic encephalitis; LEMS = Lambert-Eaton myasthenic syndrome; PCD = paraneoplastic cerebellar degeneration; PND = paraneoplastic neurological disorder; SCLC = small-cell lung cancer; SSN = subacute sensory neuronopathy.

**including 10 patients with lung cancer only on PET/CT. Other histologically confirmed cancers = non-SCLC (3), prostate adenocarcinoma (2), neuroblastoma (2), gastric adenocarcinoma (2), breast adenocarcinoma (2), small-cell bladder cancer (1), Merkel cell cancer (1), vulval endocrine cancer (1), poorly differentiated carcinoma (1).*

Supplementary Table 2. SCLC treatment regimens.

Treatment	Hu-Ab/PND/SCLC patients*	SCLC control patients	P value
Karnofsky PS<80 at time of SCLC diagnosis	19/33 (58%)[#]	101/245 (41.2%)	P=0.09
Chemotherapy (cisplatin/carboplatin and etoposide)	53/64 (83%)	220/245 (89.8%)	P=0.13
Median cycles of chemotherapy (IQR)	4 (2.5-6)	5 (3-6)	P=0.23
Thoracic radiotherapy	31/64 (48%)	111/245 (45.3%)	P=0.67
Surgical resection of tumour	3/64 (5%)	6/245 (2.4%)	P=0.4

**Immunotherapy (high dose prednisolone in 31 patients, intravenous immunoglobulin (IVIG) in 11 patients, plasma exchange in two patients, in combination) was used in 33/64 Hu-Ab/SCLC/PND patients with cancer, combined with chemotherapy/radiotherapy/tumour resection. Eight Hu-Ab/SCLC/PND patients had improvement of their PND with immunosuppression either alone (two) or in combination with anti-tumour therapy (six), although the benefit was often minimal and rarely sustained. A further five Hu-Ab/SCLC/PND patients improved neurologically after cancer therapy without immunosuppression. No patient received immune checkpoint inhibitors.*

[#]Missing data on 31 Hu-Ab/PND/SCLC patients.

Supplementary Table 3. Cox regression analysis of prognostic factors for survival in SCLC.

Prognostic factor	Hazard ratio	LCL	UCL	P value
Age <65 years	0.631	0.497	0.802	P<0.001
Female sex	0.692	0.547	0.875	P=0.002
Limited disease	0.478	0.372	0.614	P<0.001
Presence of Hu-antibody PND	0.831	0.609	1.133	P=0.242

SCLC = small-cell lung cancer; PND = paraneoplastic neurological disorder; UCL = upper 95% confidence limit of hazard ratio; LCL = lower 95% confidence limit of hazard ratio.

Data from 64 HuAb/PND/SCLC patients and 245 SCLC controls without PNDs