Case report: Immunovirotherapy as a novel add-on treatment in a patient with thoracic NUT carcinoma – Supplementary Material

Supplementary Figures

**Figure S1:** Initial pathological specimens obtained by video assisted thoracoscopy (VATS) showing the diagnosis of NC.

(A) Hematoxylin and eosin (H&E) staining: undifferentiated small round cell malignancy.

(B) Variable expression of p63.

(C) Strong nuclear reactivity with the anti-NUT monoclonal antibody.

(D) Scattered CD3-positive T lymphocytes.

(E) Main image: most T cells are CD4-positive. Subimage: very few T cells are CD8-positive.

(F) PD-L1 (SP263) found to be negative in the tumor cells, positive only in < 5% of immune cells (mainly located at the tumor-stromal-interphase).
Figure S2: Post-treatment (day 98) pathological specimen of NC obtained from the right epiphrenic tumor close to the thoracic wall.

(A) H&E: undifferentiated small round cell malignancy with partial necrosis.

(B) Variable expression of p63.

(C) Strong nuclear reactivity with the anti-NUT monoclonal antibody.

(D) Scattered CD3-positive T lymphocytes.

(E) Most T cells are CD8-positive.

(F) Very few T cells are CD4 positive (larger cells with weaker staining represent macrophages, see also (G)).

(G) CD163 highlights numerous macrophages.

(H) PD-L1 (22C3) found to be positive in <1% of tumor cells and immune cells.
Methodology

Figure 2:

(A) Tumor sequencing

TruSight RNA fusion panel sequencing (Illumina, San Diego, CA, US) for 507 fusion associated genes was conducted with a tumor specimen obtained by video assisted thoracoscopy. Integrated Genome Viewer software was used for visualization.

Figure 3:

Exact mode of radiation: 33 Gy (ICRU, International commission on radiation units and measurements) in 11 fractions, single dose 3 Gy, intensity modulated radiotherapy (IMRT) and volumetric intensity modulated arc therapy (VMAT) with 6 MV photons, image guided radiotherapy (IGRT) with cone-beam computed tomography (CB-CT).

Figure 4:

(A) HSV-1 DNA real-time Polymerase Chain Reaction (PCR)

Blood serum samples were analyzed with an HSV1&2 VZV R-GENE® PCR Kit (Argene, Biomerieux, Nürtingen) targeting the US7 HSV-1 gene. The lower limit of quantification (LLOQ) was 600 viral copies/ml. Values at “DNA detection” indicate detectable HSV-1 DNA without the possibility for quantification (<600 viral copies/ml). Values at baseline indicate no detection of HSV-1 DNA.

(C) T-VEC specific PCR

Amplification of the T-VEC transgene was performed with the polymerase OneTaq (NEB) in a total reaction volume of 20 µl according to the manufacturer’s protocol, using primers binding to

the CMV promoter sequence (GGTGGGAGGTCTATATAAGC)

or the bGH-polyA sequence (ACCTTCCAGGGTCAAGGAAG)

which flank the hGM-CSF coding sequence in the T-VEC genome. The PCR product was analyzed by gel electrophoresis on a 1% agarose gel containing ethidium bromide.

(D) Created with BioRender.com.
# Data sheet Figure 3/4

| Days (since first admission) | HSV-1 qPCR (Copies/ml) | Immuno-virotherapy (T-VEC 10^8 PFU/ml, Penciclovir ab 200 mg) | Chemotherapy | Radiation Therapy 10^3 Gy=30 Gy | IFNαC (SIAH 96 mg) | LDH (UI) |
|-----------------------------|------------------------|-------------------------------------------------|--------------|---------------------------------|-------------------|---------|
| 0                           | 0                      | x/4                                             |              |                                 |                   |         |
| 1                           | x                      | x                                               |              |                                 |                   |         |
| 2                           | x                      | x                                               |              |                                 |                   |         |
| 3                           | x                      | x                                               |              |                                 |                   |         |
| 4                           | x                      | x                                               |              |                                 |                   |         |
| 5                           | x                      | x                                               |              |                                 |                   |         |
| 6                           | x                      | x                                               |              |                                 |                   |         |
| 7                           | 70 000                 | x                                               |              |                                 |                   |         |
| 8                           | 80 000                 | x                                               |              |                                 |                   |         |
| 9                           | 165 000                | x                                               |              |                                 |                   |         |
| 10                          | 204 000                | x                                               |              |                                 |                   |         |
| 11                          | 204 000                | x                                               |              |                                 |                   |         |
| 12                          | 204 000                | x                                               |              |                                 |                   |         |
| 13                          | 204 000                | x                                               |              |                                 |                   |         |
| 14                          | x                      | x                                               |              |                                 |                   |         |
| 15                          | x                      | x                                               |              |                                 |                   |         |
| 16                          | 27 100                 | x                                               |              |                                 |                   |         |
| 17                          | x                      | x                                               |              |                                 |                   |         |
| 18                          | x                      | x                                               |              |                                 |                   |         |
| 19                          | 3 000                  | x/10                                            |              |                                 |                   |         |
| 20                          | 3 000                  | x                                               |              |                                 |                   |         |
| 21                          | 1 200                  | x                                               |              |                                 |                   |         |
| 22                          | x                      | x                                               |              |                                 |                   |         |
| 23                          | x                      | x                                               |              |                                 |                   |         |
| 24                          | x                      | x                                               |              |                                 |                   |         |
| 25                          | x                      | x                                               |              |                                 |                   |         |
| 26                          | x                      | x                                               |              |                                 |                   |         |
| 27                          | x                      | x                                               |              |                                 |                   |         |
| 28                          | 1 630                  | x                                               |              |                                 |                   |         |
| 29                          | 1 630                  | x                                               |              |                                 |                   |         |
| 30                          | 1 710                  | x                                               |              |                                 |                   |         |
| 31                          | 1 830                  | x                                               |              |                                 |                   |         |
| 32                          | 4 310                  | x                                               |              |                                 |                   |         |
| 33                          | 1 900                  | Pembin                                         |              |                                 |                   |         |
| 34                          | 2 710                  | x                                               |              |                                 |                   |         |
| 35                          | 2 710                  | x                                               |              |                                 |                   |         |
| 36                          | x                      | x                                               |              |                                 |                   |         |
| 37                          | x                      | x                                               |              |                                 |                   |         |
| 38                          | x                      | x                                               |              |                                 |                   |         |
| 39                          | <600                   | x                                               |              |                                 |                   |         |
| 40                          | <600                   | x                                               |              |                                 |                   |         |
| 41                          | <600                   | x                                               |              |                                 |                   |         |
| 42                          | <600                   | x                                               |              |                                 |                   |         |
| 43                          | <600                   | x                                               |              |                                 |                   |         |
| 44                          | <600                   | x                                               |              |                                 |                   |         |
| 45                          | 213                    | x                                               |              |                                 |                   |         |
| 46                          | 274                    | x                                               |              |                                 |                   |         |
| 47                          | x                      | x                                               |              |                                 |                   |         |
| 48                          | x                      | x                                               |              |                                 |                   |         |
| 49                          | 695                    | x                                               |              |                                 |                   |         |
| 50                          | 1 510                  | x                                               |              |                                 |                   |         |
| 51                          | 1 510                  | x                                               |              |                                 |                   |         |
| 52                          | 1 510                  | x                                               |              |                                 |                   |         |
| 53                          | 1 510                  | x                                               |              |                                 |                   |         |
| 54                          | 1 510                  | x                                               |              |                                 |                   |         |
| 55                          | 1 510                  | x                                               |              |                                 |                   |         |
| 56                          | 1 510                  | x                                               |              |                                 |                   |         |
| 57                          | <600                   | x                                               |              |                                 |                   |         |
| 58                          | <600                   | x                                               |              |                                 |                   |         |
| 59                          | <600                   | x                                               |              |                                 |                   |         |
| 60                          | <600                   | x                                               |              |                                 |                   |         |
| 61                          | Pembin                 | x                                               |              |                                 |                   |         |
| 62                          | 242                    | x                                               |              |                                 |                   |         |
| 63                          | 242                    | x                                               |              |                                 |                   |         |
| 64                          | 242                    | x                                               |              |                                 |                   |         |
| 65                          | 242                    | x                                               |              |                                 |                   |         |
| 66                          | 242                    | x                                               |              |                                 |                   |         |
| 67                          | 242                    | x                                               |              |                                 |                   |         |
| 68                          | 242                    | x                                               |              |                                 |                   |         |
| 69                          | 242                    | x                                               |              |                                 |                   |         |
| 70                          | 242                    | x                                               |              |                                 |                   |         |
| 71                          | 242                    | x                                               |              |                                 |                   |         |
| 72                          | 242                    | x                                               |              |                                 |                   |         |
| 73                          | 242                    | x                                               |              |                                 |                   |         |
| 74                          | 242                    | x                                               |              |                                 |                   |         |
| 75                          | 242                    | x                                               |              |                                 |                   |         |
| 76                          | 242                    | x                                               |              |                                 |                   |         |
| 77                          | 242                    | x                                               |              |                                 |                   |         |
| 78                          | 242                    | x                                               |              |                                 |                   |         |
| 79                          | 242                    | x                                               |              |                                 |                   |         |
| 80                          | 242                    | x                                               |              |                                 |                   |         |
| 81                          | 242                    | x                                               |              |                                 |                   |         |
| 82                          | 242                    | x                                               |              |                                 |                   |         |
| 83                          | 242                    | x                                               |              |                                 |                   |         |
| 84                          | 242                    | x                                               |              |                                 |                   |         |
| 85                          | 242                    | x                                               |              |                                 |                   |         |
| 86                          | 242                    | x                                               |              |                                 |                   |         |
| 87                          | 242                    | x                                               |              |                                 |                   |         |
| 88                          | 242                    | x                                               |              |                                 |                   |         |
| 89                          | 242                    | x                                               |              |                                 |                   |         |
| 90                          | 242                    | x                                               |              |                                 |                   |         |
| 91                          | 242                    | x                                               |              |                                 |                   |         |
| 92                          | 242                    | x                                               |              |                                 |                   |         |
| 93                          | 242                    | x                                               |              |                                 |                   |         |
| 94                          | 242                    | x                                               |              |                                 |                   |         |
| 95                          | 242                    | x                                               |              |                                 |                   |         |
| 96                          | 242                    | x                                               |              |                                 |                   |         |
| 97                          | 242                    | x                                               |              |                                 |                   |         |
| 98                          | 242                    | x                                               |              |                                 |                   |         |
| 99                          | 242                    | x                                               |              |                                 |                   |         |
| 100                         | 242                    | x                                               |              |                                 |                   |         |