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Supplemental Material to:

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The novel EpCAM-targeting monoclonal antibody 3–17I linked to saporin is highly cytotoxic after photochemical internalization in breast, pancreas and colon cancer cell lines

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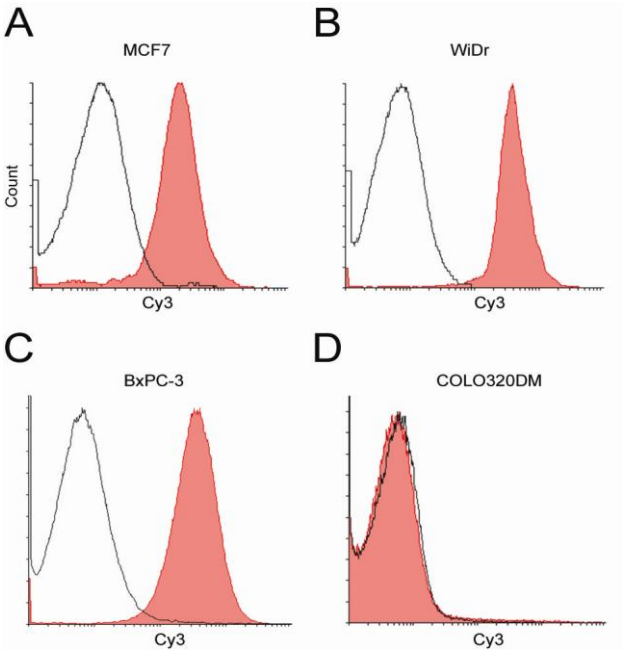
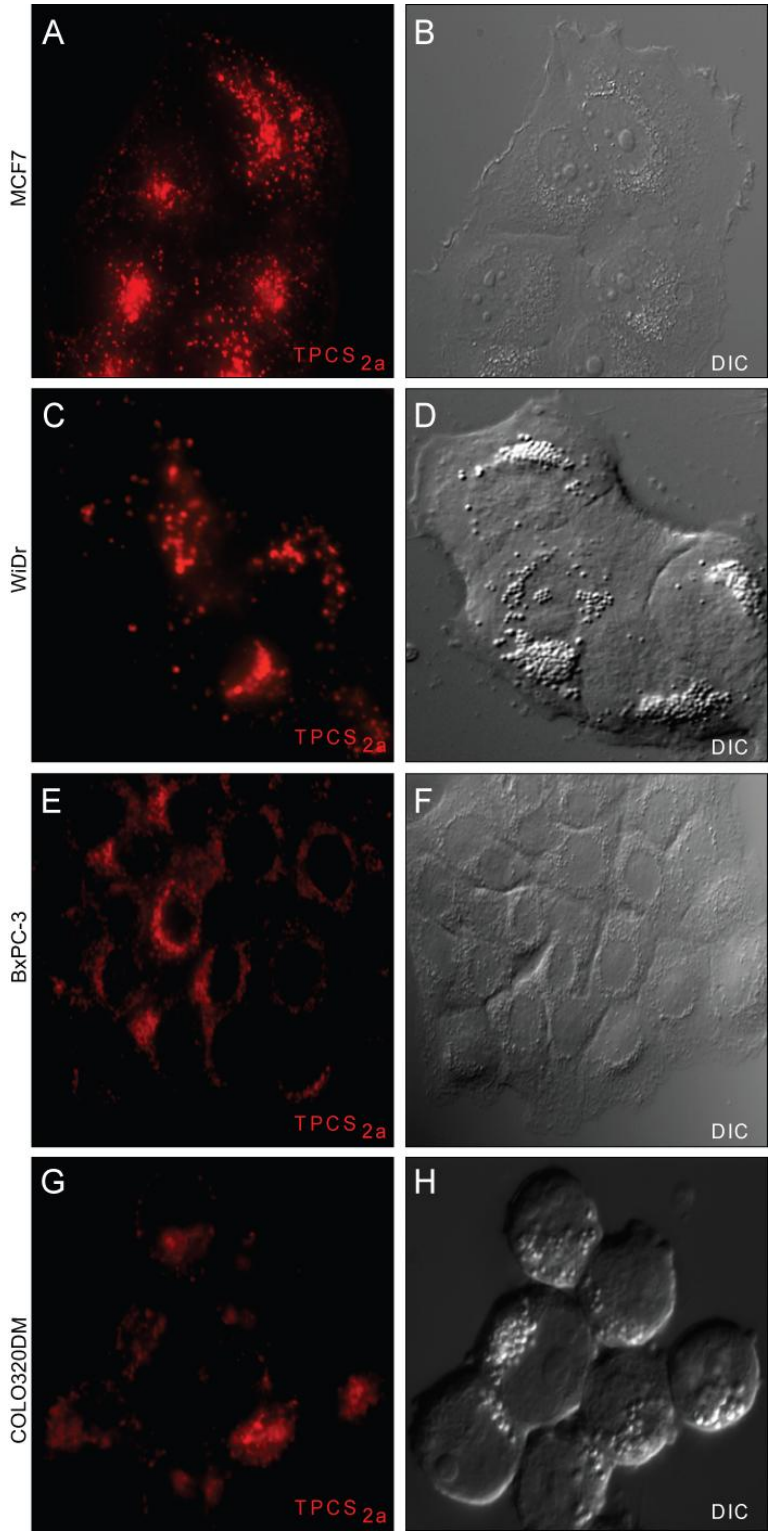


Supplementary Figure 1. Flow cytometric analysis confirms binding of biotinylated 3-17I to EpCAM-positive cell lines. The cancer cells (A) MCF7; (B) WiDr; (C) BxPC-3 and (D) COLO320DM were stained with 3-17I-CY-3 (orange), as described in *Materials and Methods.* Unstained cells (black) and the 3-17I negative cell line COLO320DM were used as a negative control.



Supplementary Figure 2. Intracellular localization of the photosensitizer ($TPCS_{2a}$/Amphinex) in carcinoma cell lines. $TPCS_{2a}$ was incubated for 18 h and chased in drug free medium for 4 h prior to epi-fluorescence microscopy, following the PCI protocol, in MCF7 (A-B); WiDr (C-D); BxPC-3 (E-F); and COLO320DM (G-H) cells.

Supplementary Table 1A. Immunohistochemistry studies of normal tissue reactivity (n = 90) of 3-17I, MT201, and MOC31 (all IgG2A), and IgG2A isotype control. Triplicate samples from 30 different tissues are included in the study. Staining intensity was judged as; negative (0), weak reaction (1+), moderate reaction (2+), or strong reaction (3+).

Tissue	MOC31B	MT201	3-17I	Isotype, IgG2A
Adrenal 1	1+, diffuse	Neg.	Neg.	Neg.
Adrenal 2	1+, diffuse	Neg.	Neg.	Neg.
Adrenal 3	Neg.	Neg.	Neg.	Neg.
Brain, cerebellum 1	Neg.	Neg.	Neg.	Neg.
Brain, cerebellum 2	Neg.	Neg.	Neg.	Neg.
Brain, cerebellum 3	Neg.	Neg.	Neg.	Neg.
Brain, cerebrum 1	Neg.	Neg.	Neg.	Neg.
Brain, cerebrum 2	Neg.	Neg.	Neg.	Neg.
Brain, cerebrum 3	Neg.	Neg.	Neg.	Neg.
Brain, pituitary, 1	3+, local	Neg.	3+, local	Neg.
Brain, pituitary, 2	3+, in epithelial cells	Neg.	3+, in epithelial cells	Neg.
Brain, pituitary, 3	3+, in epithelial cells	Neg.	3+, in epithelial cells	Neg.
Breast 1	3+, in epithelial cells	Neg.	3+, in epithelial cells	Neg.
Breast 2	3+, in epithelial cells	Neg.	3+, in epithelial cells	Neg.
Breast 3	3+, in epithelial cells	unspecific in mucin	3+, in epithelial cells	unspecific in mucin
Colon 1	3+, in epithelial cells	unspecific in mucin	3+, in epithelial cells	unspecific in mucin
Colon 2	3+, in epithelial cells	unspecific in mucin	3+, in epithelial cells	unspecific in mucin
Colon 3	3+, in epithelial cells	unspecific in mucin	3+, in epithelial cells	unspecific in mucin
Esophagus 1	2+, in epithelial cells	Neg.	3+, in sloughed cells	Neg.
Esophagus 2	2+, in epithelial cells	Neg.	Neg.	Neg.
Esophagus 3	Neg (no epithelium)	Neg.	Neg.	Neg.
Heart 1	Neg.	Neg.	Neg.	Neg.
Heart 2	Neg.	Neg.	Neg.	Neg.
Heart 3	Neg.	Neg.	Neg.	Neg.
Kidney 1	3+ in tubular epithelium	Neg.	3+ in tubular epithelium	Neg.
Kidney 2	3+ in tubular epithelium	Neg.	3+ in tubular epithelium	Neg.
Kidney 3	3+ in tubular epithelium	Neg.	3+ in tubular epithelium	Neg.

Liver 1	3+, local	1+ diffuse unspecific reaction	3+, local	Neg.
Liver 2	3+, local	1+ diffuse unspecific reaction	3+, local	Neg.
Liver 3	3+, local	1+ diffuse unspecific reaction	3+, local	Neg.
Lung 1	2+	Neg.	1+	Neg.
Lung 2	2+	Neg.	1+	Neg.
Lung 3	3+	Neg.	2+	Neg.
Skeletal muscle 1	Neg.	Neg.	Neg.	Neg.
Skeletal muscle 2	Neg.	Neg.	Neg.	Neg.
Skeletal muscle 3	Neg.	Neg.	Neg.	Neg.
Mesotheleal 1	Neg.	Neg.	Neg.	Neg.
Mesotheleal 2	Neg.	Neg.	Neg.	Neg.
Mesotheleal 3	Neg.	Neg.	Neg.	Neg.
Nerve 1	Neg.	Neg.	Neg.	Neg.
Nerve 2	Neg.	Neg.	Neg.	Neg.
Nerve 3	Neg.	Neg.	Neg.	Neg.
Ovary 1	Neg.	Neg.	Neg.	Neg.
Ovary 2	Neg.	Neg.	Neg.	Neg.
Ovary 3	Neg.	Neg.	Neg.	Neg.
Pancreas 1	3+, epithelial cells	1+ diffuse unspecific reaction	3+, epithelial cells	Neg.
Pancreas 2	3+, epithelial cells	2+, unspecific reaction	3+, epithelial cells	2+, unspecific reaction
Pancreas 3	3+, epithelial cells	2+, unspecific reaction	3+, epithelial cells	2+, unspecific reaction
Placenta 1	Neg.	Neg.	Neg.	Neg.
Placenta 2	Neg.	Neg.	Neg.	Neg.
Placenta 3	Neg.	Neg.	Neg.	Neg.
Prostate 1	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Prostate 2	Neg.(no epithelial cells)	Neg.	Neg.	Neg.
Prostate 3	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Salivary gland 1	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Salivary gland 2	3+, epithelial cells	Local unsp. reaction due to folding of section	3+, epithelial cells	Neg.
Salivary gland 3	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Skin 1	2+, in epidermis	Neg.	1+, in epidermis	Neg.
Skin 2	2+, in epidermis	Neg.	1+, in epidermis	Neg.
Skin 3	1+, in epidermis	Neg.	1+, in epidermis	Neg.
Small intestine 1	3+, epithelial cells	unspecific in mucin	3+, epithelial cells	unspecific in mucin

Small intestine 2	3+, epithelial cells	unspecific in mucin	3+, epithelial cells	unspecific in mucin
Small intestine 3	3+, epithelial cells	unspecific in mucin	3+, epithelial cells	unspecific in mucin
Spleen 1	Neg.	Neg.	Neg.	Neg.
Spleen 2	Neg.	Neg.	Neg.	Neg.
Spleen 3	Neg.	Neg.	Neg.	Neg.
Stomach 1	Neg. (no epithelium)	Neg.	3+, epithelial cells	Neg.
Stomach 2	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Stomach 3	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Testis 1	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.
Testis 2	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.
Testis 3	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.	3+, cells in seminiferous tubules, probably Sertoli cells	Neg.
Thymus 1	3+, probably in epithelial cells of medulla	1+, diffuse reaction in medulla	3+, probably in epithelial cells of medulla	Neg.
Thymus 2	3+, probably in epithelial cells of medulla	1+, diffuse reaction in medulla	3+, probably in epithelial cells of medulla	Neg.
Thymus 3	3+, probably in epithelial cells of medulla	1+, diffuse reaction in medulla	3+, probably in epithelial cells of medulla	Neg.
Thyroid 1	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Thyroid 2	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Thyroid 3	3+, epithelial cells	Neg.	3+, epithelial cells	Neg.
Tonsil 1	Neg.	Neg.	Neg.	Neg.
Tonsil 2	2+, in surrounding squamous epithelial cells	Neg.	Neg.	Neg.
Tonsil 3	2+, in surrounding squamous epithelial cells	Neg.	Neg.	Neg.
Uterus 1	Neg.	Neg.	Neg.	Neg.
Uterus 2	Neg.	Neg.	Neg.	Neg.
Uterus 3	Neg.	Neg.	Neg.	Neg.
Uterus, cervix 1	2+, in squamous epithelial cells	Neg.	Neg.	Neg.
Uterus, cervix 2	Neg. (no epithelium)	Neg.	Neg.	Neg.
Uterus, cervix 3	Neg. (no epithelium)	Neg.	Neg.	Neg.
Bone marrow 1	1+, diffuse	1+, diffuse	1+, diffuse	1+, diffuse
Bone marrow 2	1+, diffuse	1+, diffuse	1+, diffuse	1+, diffuse
Bone marrow 3	1+, diffuse	1+, diffuse	1+, diffuse	1+, diffuse

Supplementary Table 1B. 3-17I shows less reaction in healthy tissue than MOC31. Immunohistochemistry studies of 3-17I and MOC31 (both IgG2A), and IgG2A isotype control, binding to healthy tissue samples. **List summarizes the tissues in Supplementary Table 1A to which 3-17I shows less reaction than MOC31.** Staining intensity was judged as; negative (0), weak reaction (1+), moderate reaction (2+), or strong reaction (3+).

Tissue	MOC31B	3-17I	Isotype, IgG2A
Adrenal 1	1+, diffuse	Negative	Negative
Adrenal 2	1+, diffuse	Negative	Negative
Esophagus 2	2+, in epithelial cells	Negative	Negative
Lung 1	2+	1+	Negative
Lung 2	2+	1+	Negative
Lung 3	3+	2+	Negative
Skin 1	2+, in epidermis	1+, in epidermis	Negative
Skin 2	2+, in epidermis	1+, in epidermis	Negative
Thymus 1	3+, probably in epithelial cells of medulla	2+, probably in epithelial cells of medulla	Negative
Thymus 2	3+, probably in epithelial cells of medulla	2+, probably in epithelial cells of medulla	Negative
Thymus 3	3+, probably in epithelial cells of medulla	2+, probably in epithelial cells of medulla	Negative
Tonsil 2	2+, in surrounding squamous epithelial cells	Negative	Negative
Tonsil 3	2+, in surrounding squamous epithelial cells	Negative	Negative
Uterus, cervix 1	2+, in squamous epithelial cells	Negative	Negative

Supplementary Table 2A. Immunohistochemistry studies of colon cancer tissue reactivity (n=37*) of 3-17I, MOC31, MT201 (all IgG2A), and IgG2A isotype control.
Staining intensity was judged as; negative (0), weak reaction (1+), moderate reaction (2+), or strong reaction (3+).

*One colon tissue sample was omitted due to lack of malignant tumor cells.

Colon cancer tumor #	age	sex	Pathological diagnosis	MOC31B	MT201	3-17I	Isotype, IgG2A
A1	67	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A2	80	f	Adenocarcinoma	3+	Neg.	3+	Neg.
A3	59	f	Adenocarcinoma	3+	Neg.	3+	Neg.
A4	60	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A5	71	f	Adenocarcinoma	3+	Neg.	3+	Neg.
A6	46	f	Adenocarcinoma	3+	Neg.	3+	Neg.
A7	79	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A8	68	f	Adenocarcinoma mucinous	3+	Neg.	3+	Neg.
B1	37	m	Adenocarcinoma mucinous	3+	Neg.	3+	Neg.
B2	65	m	Adenocarcinoma	3+	Neg.	3+	Neg.
B3	56	f	Adenocarcinoma	3+	Neg.	3+	Neg.
B4	52	f	Adenocarcinoma	3+	Neg.	3+	Neg.
B5	63	m	Adenocarcinoma	3+	3+, in mucin	3+	3+, in mucin
B6	74	m	Adenocarcinoma	3+	Neg.	3+	Neg.
B7	75	m	Adenocarcinoma	3+	3+, unspecific	3+	3+, unspecific
B8	74	m	Adenocarcinoma	3+	Neg.	3+	Unspecific
C1	68	m	Adenocarcinoma	3+	Neg.	3+	Neg.
C2	73	m	Adenocarcinoma	3+	Neg.	3+	Neg.
C3	43	m	Adenocarcinoma	No tumor cells	No tumor cells	No tumor cells	No tumor cells
C4	64	m	Adenocarcinoma	3+	Neg.	3+	Neg.
C5	75	f	Adenocarcinoma	3+	Neg.	3+	Neg.
C6	50	m	Adenocarcinoma	3+	Neg.	3+	Neg.
C7	63	f	Adenocarcinoma	3+	Neg.	3+	Neg.
C8	61	m	Adenocarcinoma	3+	Neg.	3+	Neg.
D1	53	m	Adenocarcinoma	3+	Neg.	3+	Neg.
D2	49	f	Adenocarcinoma	3+	Neg.	3+	Neg.
D3	79	m	Adenocarcinoma	3+	Neg.	3+	Neg.
D4	70	m	Adenocarcinoma	3+	Neg.	3+	Neg.
D5	62	f	Adenocarcinoma mucinous	3+	Neg.	3+	Neg.
D6	71	m	Adenocarcinoma	3+	Neg.	3+	Neg.
D7	57	f	Adenocarcinoma	3+	Neg.	3+	Neg.
D8	87	m	Adenocarcinoma	3+	Neg.	3+	Neg.
E1	73	f	Adenocarcinoma	3+	Neg.	3+	Neg.
E2	48	m	Adenocarcinoma	3+	Neg.	3+	Neg.
E3	63	m	Adenocarcinoma	3+	Neg.	3+	Neg.
E4	36	m	Adenocarcinoma	3+	Neg.	3+	Neg.
E5	45	m	Adenocarcinoma	3+	Neg.	3+	Neg.

Supplementary Table 2B. Immunohistochemistry studies of breast cancer tissue reactivity (n=37) of 3-17I, MOC31, MT201 (all IgG2A), and IgG2A isotype control. Staining intensity was judged as; negative (0), weak reaction (1+), moderate reaction (2+), or strong reaction (3+).

Breast cancer tumor #	age	sex	Pathological diagnosis	MOC31B	MT201	3-17I	Isotype, IgG2A
A1	64	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A2	70	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A3	48	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A4	57	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A5	65	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A6	65	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A7	53	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
A8	82	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B1	64	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B2	34	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B3	40	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B4	48	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B5	57	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B6	77	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B7	42	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
B8	47	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C1	34	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C2	48	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C3	41	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C4	47	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C5	37	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C6	32	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C7	63	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
C8	50	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D1	56	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D2	56	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D3	41	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D4	47	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D5	60	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D6	46	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D7	45	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
D8	57	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
E1	37	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
E2	57	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
E3	67	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
E4	63	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.
E5	50	f	Invasive ductal adenocarcinoma	3+	Neg.	3+	Neg.

Supplementary Table 2C. Immunohistochemistry studies of lung cancer tissue reactivity (n=37*) of 3-17I, MOC31, MT201 (all IgG2A), and IgG2A isotype control.
Staining intensity was judged as; negative (0), weak reaction (1+), moderate reaction (2+), or strong reaction (3+).

*One lung tissue sample was a sarcoma and thus has no epithelial origin.

Lung cancer tumor #	age	sex	Pathological diagnosis	MOC31B	MT201	3-17I	Isotype, IgG2A
A1	39	f	Adenocarcinoma	3+	Neg.	3+	Neg.
A2	66	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A3	70	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A4	70	m	Adenocarcinoma	3+	Neg.	3+	Neg.
A5	64	f	Adenocarcinoma, papillary	3+	Neg.	3+	Neg.
A6	68	m	Undifferentiated small cell carcinoma	3+	Neg.	3+	Neg.
A7	39	m	Undifferentiated small cell carcinoma	3+	Neg.	3+	Neg.
A8	75	m	Bronchio alveolar carcinoma	3+	Neg.	3+	Neg.
B1	50	m	Bronchio alveolar carcinoma	3+	Neg.	3+	Neg.
B2	42	m	Bronchio alveolar carcinoma	3+	Neg.	3+	Neg.
B3	69	m	Carcinoma, sarcoma	Neg.	Neg.	Neg.	Neg.
B4	61	m	Carcinoma, sarcoma todes	3+	Neg.	3+	Neg.
B5	60	m	Harmatoma	3+	Neg.	3+	Neg.
B6	46	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
B7	63	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
B8	53	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C1	69	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C2	64	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C3	33	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C4	62	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C5	66	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C6	63	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C7	53	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
C8	53	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D1	70	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D2	73	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D3	68	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D4	65	f	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D5	50	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D6	52	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D7	66	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
D8	72	f	Squamous cell carcinoma	3+	Neg.	3+	Neg.
E1	57	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
E2	66	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
E3	70	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
E4	62	m	Squamous cell carcinoma	3+	Neg.	3+	Neg.
E5	61	f	Squamous cell carcinoma	3+	Neg.	3+	Neg.