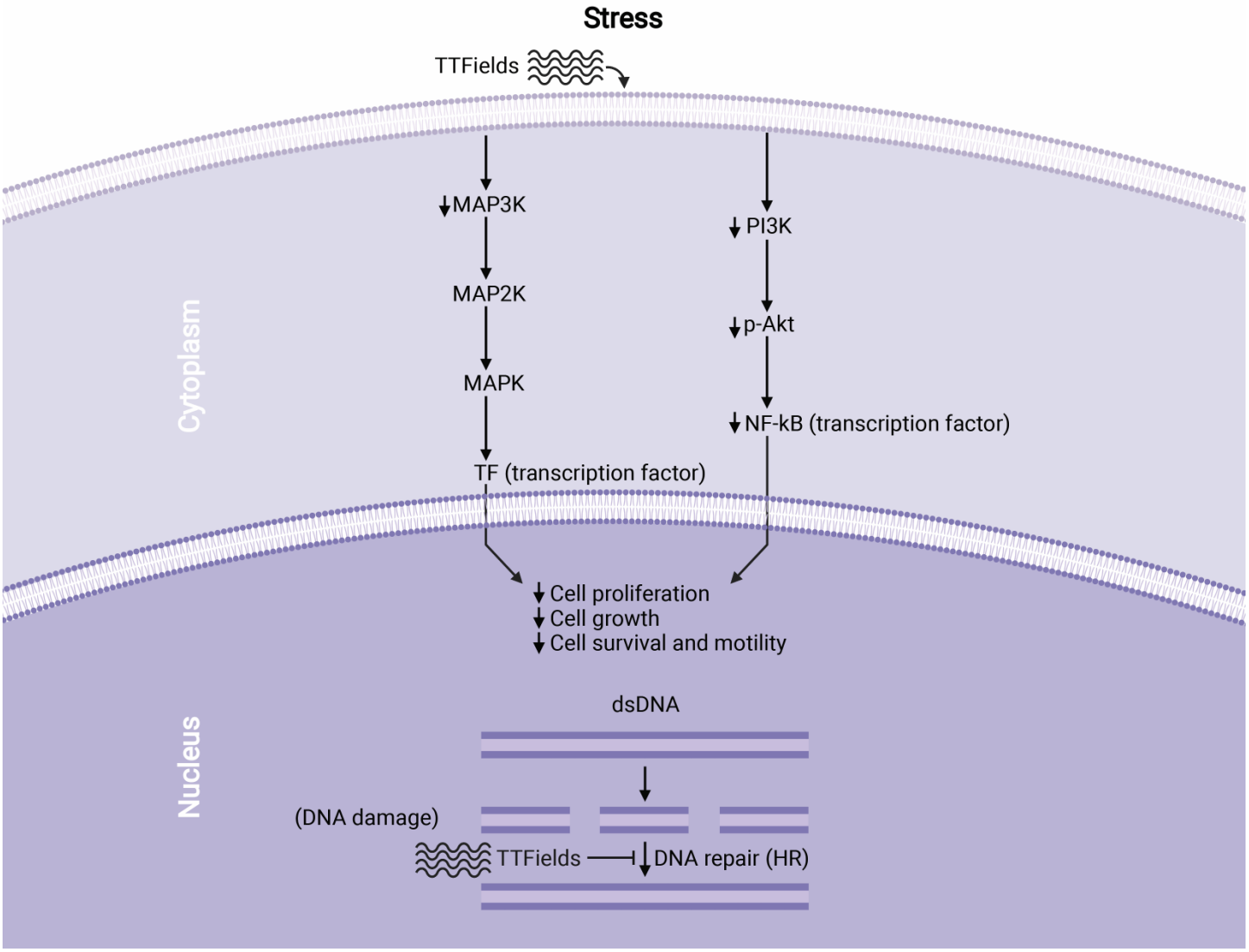
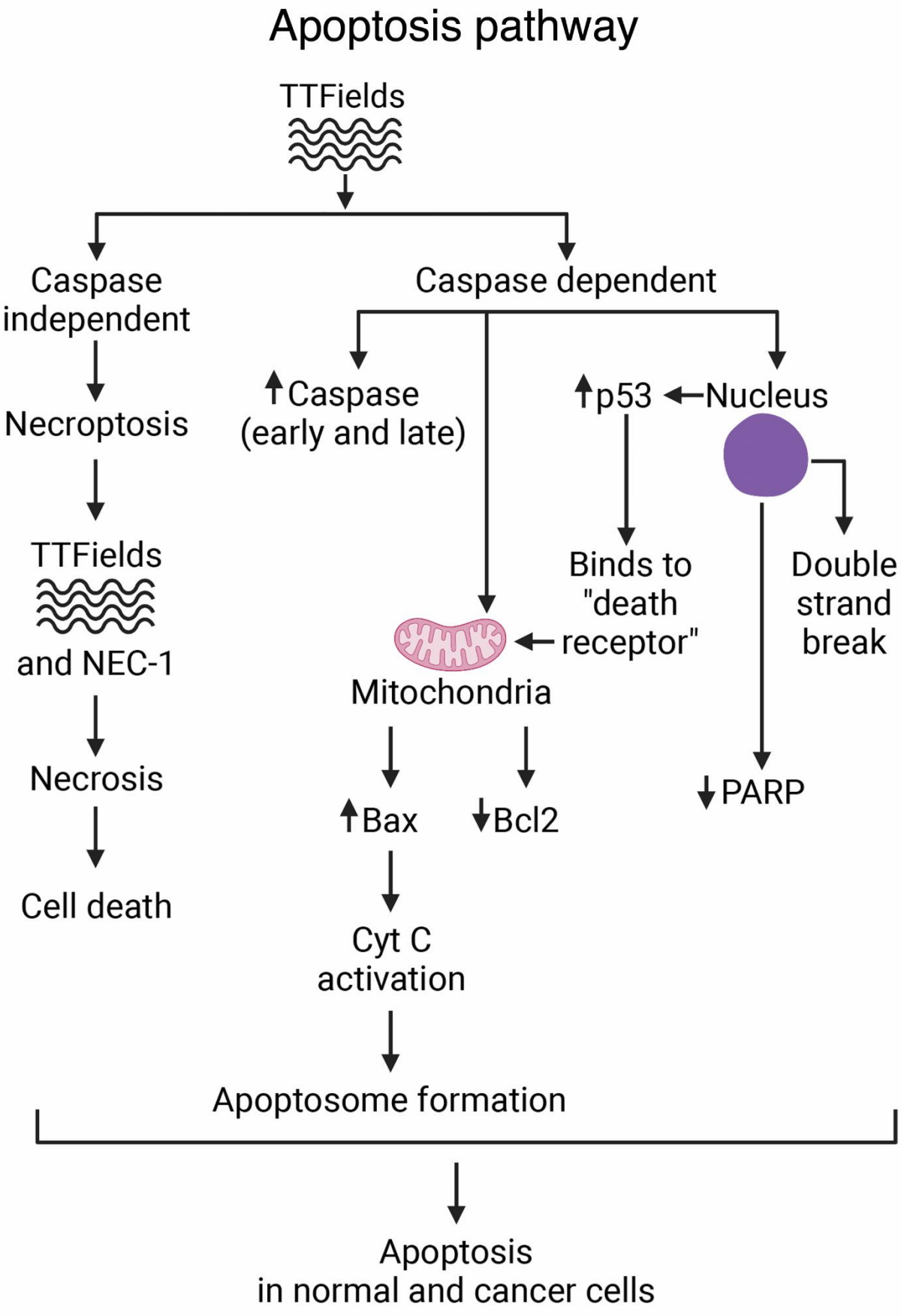
Supplementary materials (3 supplementary figures and 4 supplementary tables)

S. Shams and C. B. Patel. Anti-cancer mechanisms of action of therapeutic alternating electric fields (tumor treating fields [TTFields]). Journal of Molecular Cell Biology, mjac047, https://doi.org/10.1093/jmcb/mjac047 . Published: 16 August 2022

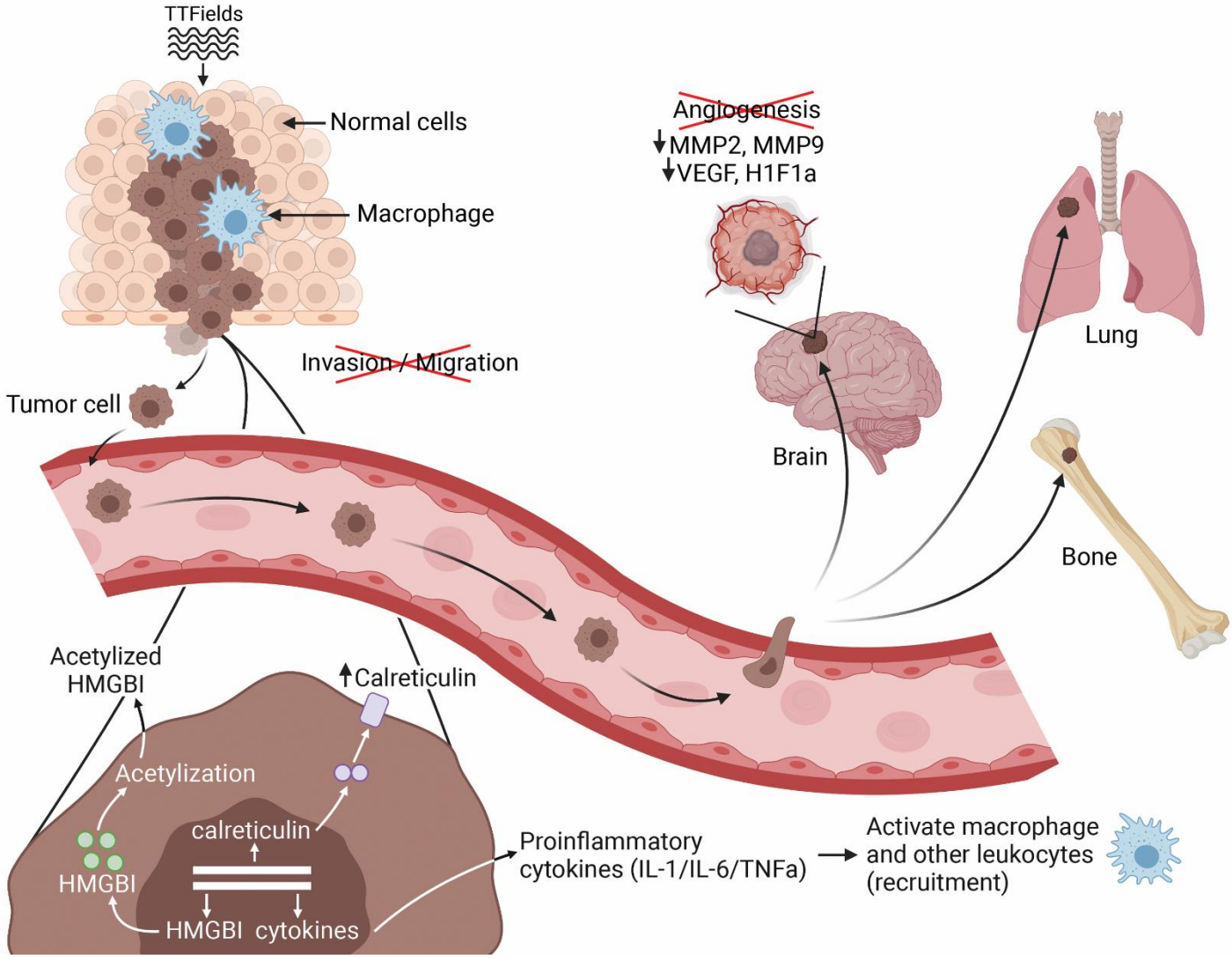
Supplementary materials available online:
https://academic.oup.com/jmcb/advance-article/doi/10.1093/jmcb/mjac047/6668799#supplementary-data



Supplementary Figure 1: TTFields hinders cancer cell growth and proliferation through interference with normal intracellular signaling pathways. TTFields induced alteration in the cytosolic signaling pathways, combined with its intranuclear DNA damaging effects and interference with DNA damage repair, lead to reduced cancer cell proliferation, growth, survival, and motility.



Supplementary Figure 2: TTFields induces cancer cell death via caspase independent (e.g., necroptosis) and caspase dependent pathways.



Supplementary Figure 3: TTFields exposure interferes with cancer cell mediated angiogenesis, impairs invasion and migration (thereby potentially reducing the likelihood of metastasis), and upregulates proinflammatory signaling and immune cell recruitment (which further aids in detecting and clearing cancer cells from adjacent non-cancer tissues).

Supplementary Table 1: Optimal anti-proliferative TTFields frequencies for various cancer cell types as reported in the literature

Cell line(s) studied	TTFields frequency (kHz)
Human glioblastoma (U87-MG*, A172, LN229**, U-118MG, NCI-H2052, GaMG, T98G, U251)	200 (*also 100 and 150; **also 100)
Human glioblastoma cells (MZ-54)	250
Human glioblastoma cells (GBM2, GBM39)	240
Human long-term glioma cell (LTC) (LN-18, LN-428, LN-319, A172, T98G, LN-308)	100
Human glioblastoma (KNS42)	200
Human glioblastoma (SF188)	400
Human glioblastoma (GIN-31)	200
Human glioblastoma (U373)	150
Human glioblastoma (2H11)	150
Human Medulloblastoma (DAOY)	300
Human Medulloblastoma (UW228-3)	100
Human Ependymoma (DKFZ-EPN1)	100
Human Ependymoma (BXD-1425EPN)	200
Human Non-small Cell Lung Cancer (H1299)	100, 150
Human Non-small Cell Lung Cancer (H157)	100
Human Non-small Cell Lung Cancer (H1650)	100
Human squamous cell carcinoma (HTB-182)	150
Human adenocarcinoma (HCC827)	150
Human Non-small Cell Lung Cancer (H4006)	150
Lung Adenocarcinoma (A549)	150, 200
Human fibroblast (PCS-201)	200
Human breast carcinoma (MDA-MB231)	150
Human breast cancer (BT-549)	150
Human breast adenocarcinoma (MCF-7)	150
Human breast ductal carcinoma (JIMT-1 and BT-474)	150
Mouse melanoma (B16F1)	100
Human non-small-cell lung carcinoma (H1299)	200
Rat glioma (F-98)	150
Human biphasic mesothelioma (MSTO-211H) and NCI-H2052	150
Murine squamous cell carcinoma (KLN-205)	150
Murine Lewis lung carcinoma (LLC-1)	150
Human pancreatic adenocarcinoma (AsPC-1, BxPC-3, BxGem)	150
Nonmalignant human hTERT-HPNE immortalized pancreatic duct cell line CRL-4023	150
Hamster pancreatic adenocarcinoma (PC-1.0)	200
Human ovarian carcinoma (A2780)	200
Human ovarian adenocarcinoma (OVCAR-3 (HTB-161) and Caov-3 (HTB-75))	150
Human cervical adenocarcinoma HeLa (Tubulin-GFP)	120, 150, 200

Cervical adenocarcinoma (HeLa)	150
Patient-derived glioblastoma stem cells (528NS and 83NS) in vivo primary cells	200
rat glioma BT4Ca	100
Human GIC (ZH-161, T-325, T-269, S-24, ZH-305)	150
Murine mammary carcinoma cells (4T1)	150
Human melanoma (A375SM)	150
Human skin normal cells (CCD-986sk)	150
Mouse melanoma cells (B16F10)	150
Mouse embryo cells (NIH3T3)	150
Human colorectal cancer (HCT116, HT29, SW620)	200
GBM stem-like cells (GSCs): MGMT expressing (TMZ resistant: 12.1 (rGBM) and 22 GSC) and non-MGMT expressing (TMZ sensitive: 33 and 114 GSC)	150
Lewis lung carcinoma (LLC-1)	200
Murine colon carcinoma (CT-26)	200
Spontaneously transformed murine ovarian surface epithelial (MOSE-L)	150
Human hepatocellular carcinoma (HEPG2)	150
Human lung squamous cell carcinoma (H520)	150
Bone marrow-derived Dendritic Cells (BMDCs)	150
Human umbilical vein endothelial cells (HUVECs)	150
Murine squamous cell carcinoma (KLN205)	150
A clonal derivative (C11) of parental Chinese hamster ovary AA8 cells and their emetine-resistant sub-line EmtR1	150 (6.58 V/cm), 200 (1.33 V/cm)
Yeast cells with fluorescence tagged (Nop65)	120
Normal Lung fibroblast (HEL299)	120
Hepatocellular carcinoma (Huh7)	150
Normal intestinal epithelial (IEC-6)	150
Human Osteosarcoma (U2OS, KHOS/NP)	150
Murine endothelial (2H11)	150
Pancreatic Cancer cell lines (CFPAC-I and HPAF-II)	200
Bone marrow derived dendritic cells (BMDCs) and splenic T-cells	200
Glioblastoma Stem Cells (NCH644, GBM1, BTSC233, JHH520, SF188)	200
Peripheral blood mononuclear cell (PBMC)	200
CAG multiple myeloma	200

Supplementary Table 2: Combination therapies used with TTFields in various cancer cell lines (organized based on the concomitant treatment used)

PMID	First Author	Year of Publication	Concomitant Treatment (Drug or Radiation)	Dose
31375748	Kim EH	2019	Chloroquine	20 mM
32144446	Voloshin T	2020	Chloroquine	20 μM
30341282	Shteingauz A	2018	Chloroquine	1-27 μM (20 μM for most experiments
30341282	Shteingauz A	2018	Vinblastine	25 nM
26658786	Giladi M	2016	Vinorelbine	30 nM
26658786	Giladi M	2016	Caspase inhibitor (Z-VAD-FMK)	20 μM
24555979	Giladi M	2014	Gemcitabine	0-100 nM
24555979	Giladi M	2014	Irinotecan	0-100 nM
31842288	Lee YJ	2019	5 Fluorouracil (5-FU)	5 μM
24555979	Giladi M	2014	5 Fluorouracil (5-FU)	0-1,000 nM
29284495	Giladi M	2017	Bleomycin	100 μg/mL
28425987	Silginer M	2017	3-methyladenine (3-MA)	1 mM
31375748	Kim EH	2019	3-methyladenine (3-MA)	2 mM
30469352	Jo Y	2018	Sorafenib	0-20 μM
33163284	Kim JY	2020	Sorafenib	0-20 μM
30210815	Kessler AF	2018	Spindle assembly checkpoint inhibitor (IN-3)	4 μM
30669316	Neuhaus E	2019	Benidipine	1 μM
30669316	Neuhaus E	2019	Nifedipine	1 μM
25213867	Giladi M	2014	Cisplatin	0.1-100 nM
34482104	Mumblat H	2021	Cisplatin	80 nM - 10 μM
31707040	Karanam NK	2020	Cisplatin	H1299 (2 μM), A549 (2 μM), H157 (1 μM), H4006 (0.75 μM)
31707040	Karanam NK	2020	Olaparib	1, 5, 10, 20, and 40 μM
27561100	Voloshin T	2016	Paclitaxel	1-100 nM
25213867	Giladi M	2014	Paclitaxel	0.1-100 nM
26658786	Giladi M	2016	Paclitaxel	33 nM
19133110	Kirson ED	2009	Paclitaxel	5.00 nM
19133110	Kirson ED	2009	Paclitaxel	0.01-1000 nM
20492723	Schneiderman RS	2010	Paclitaxel	MDA: 5 nM; MCF7: 0.1 μM; AA8: 0.1 μM
26010837	Gera N	2015	Paclitaxel	3 μM
34482104	Mumblat H	2021	Paclitaxel	2 nM - 4 μM
28681243	Chang E	2017	Doxorubicin	0.1 - 100 μM
20492723	Schneiderman RS	2010	Doxorubicin	30 μM
30350219	Lei KF	2018	Doxorubicin	0.01 - 10 μM
20492723	Schneiderman RS	2010	Doxorubicin	MDA (0.04 μM), MCF7 (0.5 μM), AA8 (0.6 μM)
19133110	Kirson ED	2009	Doxorubicin	0.001-10 μM
19133110	Kirson ED	2009	Cyclophosphamide	0.0001-100 mM
19133110	Kirson ED	2009	Doxorubicin	0.04 μM
19133110	Kirson ED	2009	Cyclophosphamide	6.60 mM

19133110	Kirson ED	2009	Light activated dacarbazine	0.001-100 mM
28425987	Silginer M	2017	Staurosporine	1 μM
28425987	Silginer M	2017	Caspase inhibitor (Z-VAD-FMK)	10 μM
28425987	Silginer M	2017	necrostatin-1 (Nec-1)	100 μM
27865821	Clark PA	2017	Temozolomide (TMZ)	0.1-1,000 μM
33371210	Vargas-Toscano A	2020	Temozolomide (TMZ)	0 - 160 μM
28425987	Silginer M	2017	Temozolomide (TMZ)	LN-18 (100 μM), LN-229 (5 μM), T-325 (200 μM), ZH-161 (25 μM)
31401938	Park J	2019	Lipopolysaccharide	1 ng/mL
28681243	Chang E	2017	Withaferin A	0.1 μM
25213867	Giladi M	2014	Pemetrexed	0-1 nM
25213867	Giladi M	2014	Erlotinib	0-100 nM
32054945	Yoon YN	2020	Barium titanate nanoparticles (BTNPs), size 100-200 nm	0-100 μg/mL
32054945	Yoon YN	2020	Cytochalasin D	150 nM
32054945	Yoon YN	2020	Amiloride	400 μM
33371210	Vargas-Toscano A	2020	Rapalink 1	0.42 nM
29284495	Giladi M	2017	Radiation therapy	0-8 Gy (4 Gy for most experiments)
31707040	Karanam NK	2020	Radiation therapy	1, 2, and 4 Gy
28425987	Silginer M	2017	Radiation therapy	LN-18 and LN-229 (3 Gy); T-325 and ZH-161 (5 Gy)
28358361	Karanam NK	2017	Radiation therapy	2 and 4 Gy
30302280	Jo Y	2019	Radiation therapy	2 Gy
33074042	Jo Y	2020	Radiation therapy	5 Gy
34395289	Linder B	2021	Dexamethasone	10 g/L
34522459	Kim JS	2021	Trastuzumab	5 μg/mL (total 100 μg)
34804927	Bai L	2021	Hyperthermia	mild hyperthermia 38.5 C
35411245	Jo Y	2022	Hyperthermia	41 C for 30 min/day

Supplementary Table 3: TTFields combination therapies in animal models of cancer (organized based on the concomitant treatment used with TTFields)

PMID	First Author	Year of Publication	Concomitant Treatment (Drug or Radiation)	Dose
24555979	Giladi M	2013	5FU	45 mg/kg
			Gemcitabine	2.5 mg/kg
25955102	Castellvi Q	2015	Gemcitabine	100 mg/kg
30469352	Jo Y	2018	Sorafenib	30 mg/kg
33163284	Kim JY	2020	Sorafenib	30 mg/kg
27561100	Voloshin T	2016	Paclitaxel	20 mg/kg
25213867	Giladi M	2014	Paclitaxel	20 mg/kg
19133110	Kirson ED	2009	Paclitaxel	20 mg/kg
32144446	Voloshin T	2020	anti-PD-1 (αPD-1) or Rat IgG2a	250 μg/mouse
25213867	Giladi M	2014	Pemetrexed	1 mg/kg
			Cisplatin	5 mg/kg
34482104	Mumblat H	2021	Pemetrexed	5 or 100 mg/kg
			Cisplatin	1 or 4 mg/kg
31842288	Lee YJ	2019	5-FU	30 mg/kg
34522459	Kim JS	2021	Trastuzumab	150 μg
29284495	Giladi M	2017	Radiation therapy	2 Gy
34659907	Lee WS	2021	Radiation therapy	3 Gy

Supplementary Table 4: Summary of the clinical trials studying TTFields (* = completed trial)

Status or Phase	Clinical trial name	Cancer type	TTFields frequency (kHz)	Combination therapy
Phase 2	*EF-07	Newly-diagnosed and recurrent glioblastoma	200	Temozolomide or N/A
	*EF-15	Advanced non-small cell lung cancer	150	Pemetrexed
	*EF-20 (PANOVA)	Advanced pancreatic adenocarcinoma	150	Gemcitabine (± Nab-paclitaxel)
	*EF-22 (INNOVATE)	Recurrent ovarian carcinoma	200	Paclitaxel
	*EF-30 (HEPANOVA)	Advanced hepatocellular carcinoma	150	Sorafenib
	EF-31	Unresectable gastroesophageal junction or gastric adenocarcinoma	150	Oxaliplatin + capecitabine (± trastuzumab)
	EF-33	Recurrent glioblastoma	200	N/A (high density TTFields transducer arrays)
	EF-36 (KEYNOTE B36)	Advanced non-small cell lung cancer	150	Pembrolizumab
Phase 3	EF-24 (LUNAR)	Stage 4 non-small cell lung cancer following platinum failure	150	Immune checkpoint inhibitors or docetaxel
	EF-25 (METIS)	1-10 brain metastases from non-small cell lung cancer	150	Radiosurgery
	EF-27 (PANOVA-3)	Locally-advanced pancreatic adenocarcinoma	150	Gemcitabine + Nab-paclitaxel
	EF-28 (INNOVATE-3)	Recurrent ovarian carcinoma	200	Paclitaxel
	EF-32 (TRIDENT)	Newly-diagnosed glioblastoma	200	Radiation + temozolomide
FDA approved	*EF-11	Recurrent glioblastoma	200	N/A
	*EF-14	Newly-diagnosed glioblastoma	200	Temozolomide
	*EF-23 (STELLAR)	Malignant pleural mesothelioma	150	Pemetrexed + (cisplatin or carboplatin)