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Supplementary Materials for

Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-^{13}C]Pyruvate

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SUPPLEMENTARY METHODS

Generation of ^{13}C Agent

The hyperpolarized [1-^{13}C]-pyruvate was produced under aseptic conditions using a DNP polarizer located in a clean room adjacent to the 3 T MR scanner. After dissolution and neutralization, column filtration removed the EPA, passed the hyperpolarized agent through a 0.22-mm sterile filter and collected it in a drug product vessel. The automated QC system provided by GE Healthcare required 3 ml for testing. An investigational pharmacist (M.F. or his designee) monitored the process and released the sample for injection if it met the specifications defined in the IND: pH in the range of 6.7-8.0, temperature in the range 25-37°C, polarization not less than 15% and residual EPA concentration no higher than 3.0 µg. The drug product vessel was then transferred to the scan room and the appropriate volume drawn into a syringe for manual injection. Concentrations of the agent and volumes delivered for each subject are given in table S1.

Determination of maximum dose

The choice of the maximum dose of hyperpolarized [1-^{13}C]-pyruvate was based upon two previous studies that had been performed by GE Healthcare in normal volunteers using injections of ^{12}C-pyruvate, but without imaging. The first of these was a phase 1, placebo-controlled study designed to determine whether there was evidence of significant adverse events (AEs) for doses up to 0.71 ml/kg in healthy volunteers between the ages of 18 and 45 years. The plan was for 4/6 subjects at each dose to receive injections of ^{12}C pyruvate and 2/6 to receive injections of saline. Up to 0.43 ml/kg body weight, the agent was well-tolerated with no AEs of concern.

For the cohort receiving a dose of 0.57 ml/kg body weight of ^{12}C-pyruvate, it was found that 2/6 subjects showed AEs that were not serious, but were of concern. The study was unblinded and the events were found to occur in subjects who had received an injection of ^{12}C-pyruvate. After review by medically

qualified representatives, it was decided not to proceed with the next planned dose level of 0.71 ml/kg and to amend the protocol in order to repeat the 0.43 ml/kg dose level. The data from this second cohort showed that the agent was well tolerated. Serum biochemistry and post-dosing changes were considered unremarkable for all participants, and there were no notable changes in vital signs, hematology, urinalysis, electrocardiogram (EKG) or other safety variables.

In a second phase 1, placebo-controlled study used doses of ^{12}C-pyruvate up to 0.43 ml/kg body weight in 30 elderly volunteers who were 60 or more years of age. No significant or serious AEs were observed and changes that did occur were mild in intensity, short-lasting and resolved spontaneously without treatment or intervention. As had been the case for the previous study, data on serum biochemistry variables and post-dosing changes were unremarkable, with no notable changes in vital signs, hematology, EKG or other safety variables.

Multiparametric MR staging examination

Multiparametric MR examinations have been widely applied to evaluate patients with prostate cancer (*44*). These include T_1-weighted and T_2-weighted fast spin echo anatomic images, diffusion-weighted images, and ^{1}H spectroscopic images. The diffusion images were processed using software available on the scanner to generate a map of apparent diffusion coefficient. The spectroscopic imaging data were reconstructed and quantified using software developed in our laboratory, which is available as an open source package for other institutions to download and modify (*48*). Areas suspected to be tumor on the basis of the staging examination were highlighted for comparison with the results from the ^{13}C examination and from prior biopsies.

SUPPLEMENTARY FIGURE

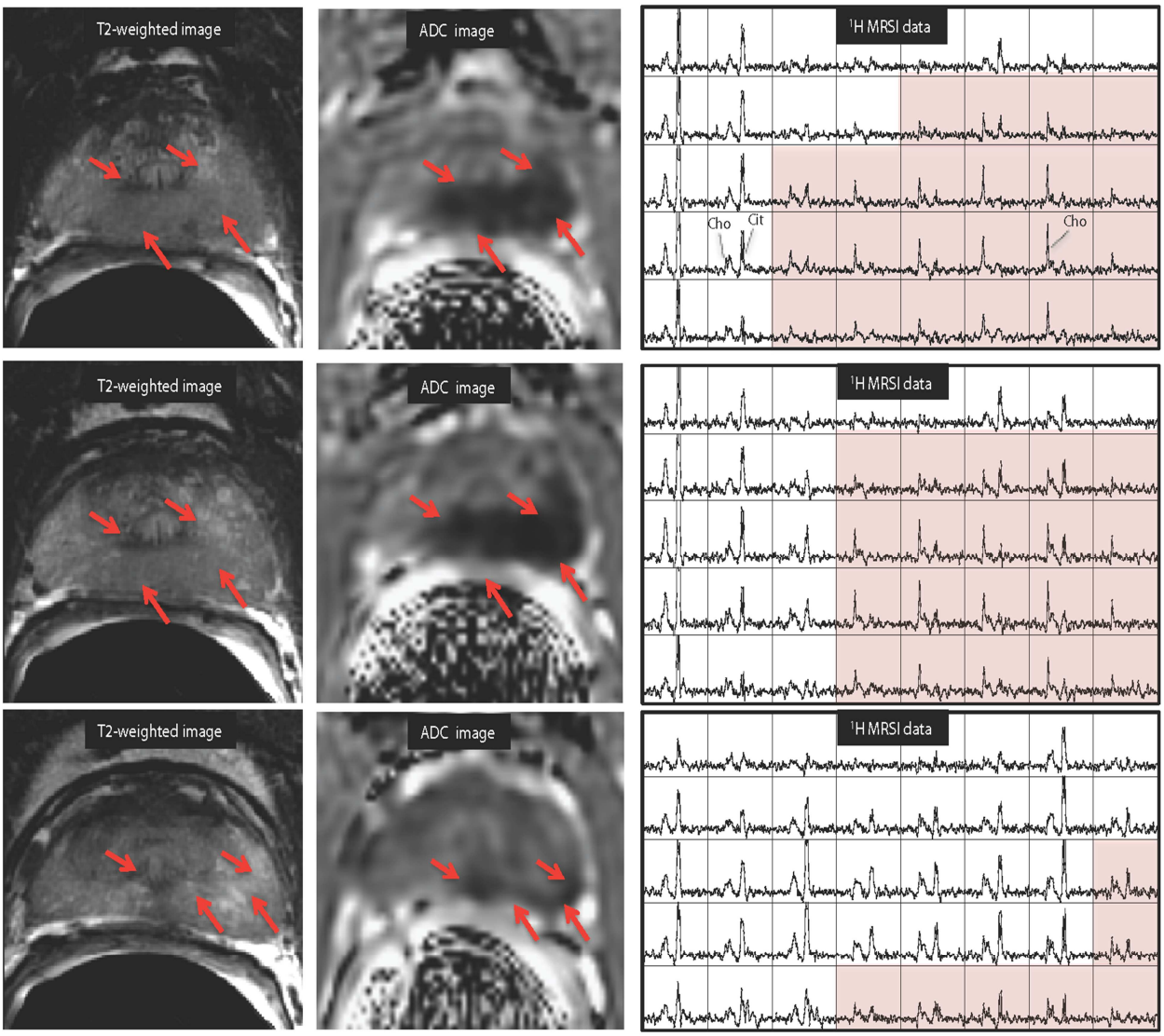

Fig. S1. Results from the MR staging examination for a patient with a large volume of bilateral cancer. The results show the T_2-weighted anatomic images, the apparent diffusion coefficient (ADC) and the ^{1}H MRSI data from the MR staging examination that was performed prior to the hyperpolarized ^{13}C examination from 3 slices through the prostate gland for the patient whose ^{13}C data were shown in Fig. 5 and in Table 2.

SUPPLEMENTARY TABLES

Table S1. Summary of information about individual patients. These include the volume of agent used, the values obtained for QC tests, the times taken to complete for each step of the preparation and the type of MR data acquired for each patient

Patient #	Agent Information			QC criteria				Timings					Type of MR data
	Dose level (ml/kg)	[Pyruvate] (^{13}C NMR) (mM)	Pyruvate delivered (ml)	pH	Polarization	Volume (ml)	Temperature (°C)	Dissolution (s)	Quality control (s)	Hatch (s)	Injection (s)	Overall time (s)	
1	0.14	225.4	10	8.0	15.9	49.3	28.8	17	13	16	6	52	1D Dynamic
2	0.14	218.5	12	7.8	17.8	31.9	31.9	5	19	11	8	43	1D Dynamic
3	0.14	250.9	14	7.7	16.3	52.4	30.5	20	11	18	9	58	1D Dynamic
4	0.14	232.7	13	7.6	15.9	52.3	32.9	30	13	16	10	69	3D MRSI
5	0.14	242.6	11	7.5	16.4	53.2	29.2	21	15	17	9	62	3D MRSI
6	0.14	203.5	11	7.6	18.2	52.8	33.7	19	13	17	9	58	3D MRSI
7	0.28	250.1	26	7.4	18.8	52.0	33.0	22	10	18	10	60	1D Dynamic
8	0.28	228.9	22	7.6	18.2	52.4	32.8	6	12	21	10	49	1D Dynamic
9	0.28	217.2	31	7.8	17.6	53.8	32.3	21	13	27	27	88	1D Dynamic
10	0.28	240.6	33	7.6	16.2	53.0	31.3	22	13	18	18	71	3D MRSI
11	0.28	222.2	24	8.0	16.0	53.0	31.4	16	15	20	14	65	3D MRSI
12	0.28	253.5	25	7.4	18.0	52.8	35.0	17	14	18	10	59	3D MRSI
13	0.43	237.3	29	7.3	16.3	53.8	33.8	17	12	21	11	61	1D Dynamic
14	0.43	244.5	32	7.6	16.3	50.6	31.5	17	13	31	16	77	1D Dynamic
15	0.43	254.1	41	7.4	17.3	53.8	32.5	16	12	35	16	79	1D Dynamic
16	0.43	224.9	33	7.7	16.6	52.8	33.4	18	12	24	13	67	2D MRSI
17	0.43	231.1	40	7.6	16.1	53.0	35.2	18	11	26	17	72	2D MRSI
18	0.43	227.7	41	7.8	17.4	53.2	34.9	17	11	22	28	78	2D MRSI
19	0.43	236.5	43	7.6	20.2	52.9	30.9	16	11	30	14	71	2D MRSI
20	0.43	250.8	46	7.6	21.1	53.0	34.4	16	18	22	20	76	2D MRSI

21	0.43	238.4	36	7.5	18.7	52.4	33.9	18	13	24	14	69	2D MRSI
22	0.43	224.8	40	7.8	14.5	53.2	31.8	23	11	21	21	76	2D Dynamic
23	0.43	234.5	35	7.5	19.6	53.6	32.5	19	11	20	14	64	2D Dynamic
24	0.43	230.8	45	7.5	18.9	53.2	30.5	17	12	21	21	71	3D MRSI
25	0.43	214.4	38	7.5	18.7	52.6	33.5	16	11	20	16	63	2D Dynamic
26	0.43	221.8	40	7.6	18.4	52.8	29.6	17	13	21	28	79	3D MRSI
27	0.43	258.3	31	7.6	19.3	50.7	32.5	17	12	23	13	65	3D MRSI
28	0.43	219.1	46	7.5	19.3	51.5	30.4	17	14	24	19	74	3D MRSI
29	0.43	232.0	30	7.6	18.6	53.5	33.2	21	12	21	12	66	3D MRSI
30	0.43	233.0	37	7.4	18.7	51.5	30.0	20	18	26	15	79	2D Dynamic
31	0.43	214.7	40	7.8	19.8	52.8	36.4	17	18	26	14	75	3D MRSI
Average		233.3	30.5	7.6	17.7	51.9	32.2	17.9	12.9	21.6	14.9	67.4	
SD		13.5	11.3	0.2	1.5	3.8	1.9	4.4	2.3	4.9	5.7	9.9	

Table S2. Number of adverse events that were observed and their grade as defined by criteria from the National Cancer Institute (*45*). Note that only events of grade 2 or higher that were attributed to the imaging agent would have been considered as dose limiting and none were observed in this study. On independent review of the records the events marked with an asterisk (*) were attributed to causes other than the hyperpolarized injection.

Study component	**Dose level**	***n* patients total**	***n* patients with events**	***n* events total**	**Symptom reported**	***n* events**	**Grade**
Phase 1	0.14 ml/kg	6	3	3	Orange urine	1	1
					Pharmaceutical smell	1	1
					Pruritus	1	1
	0.28 ml/kg	6	2	2	Cold sensation with injection	1	1
					Dysgeusia (distorted taste)	1	1
	0.43 ml/kg	6	3	5	Sore throat	1	1
					Hypocalcemia	1	1
					Hypokalemia	1	1
					Dysgeusia (distorted taste)	2	1
Phase 2	0.43 ml/kg	13	5	10	Dizziness*	1	1
					Dysgeusia (distorted taste)	2	1
					Fatigue	1	1
					Hypotension	1	1
					Nausea	1	1
					Pain (headache)	1	1
					Smell Change	2	1
					Diarrhea*	1	2