Pain Reduction in Validated Animal Pain Models: Radio Frequency Energy (RFE) targeted at the ultra-low end of the RFE spectrum (*ul*RFE®) Using the Voyager Delivery System

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Background: EMulate Therapeutics, Inc. has developed a platform medical device technology to deliver specific Radio Frequency Energy targeted at the ultra-low end of the RFE spectrum (*ul*RFE®) that simulate biological effects of molecules. EMulate calls the *ul*RFE® signals "cognates". Past tests in oncology cell culture studies [1] and clinical trials [2,3] have provided proof of concept and translation into clinical trials for newly diagnosed and recurrent GBM and pediatric Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma (DIPG/DMG). Here we present the results of a screen for pain reduction using selected *ul*RFE® signals developed by EMulate Therapeutics, Inc. and the confirmatory assays on sub-sets of signals

Methods: The pain screen and the confirmatory assays were done by an independent CRO (ANS Biotech SA, RIOM Cedex, FRANCE) using their proprietary screening system (ALGOGram™) and traditional pain assessment assays, powered for statistical analysis. All rats were exposed to a signal, continuously, for 24 hours.

ANS runs a drug screen system (N=4 rats per pain model and cognate) and compares the outcomes from historical data (they call this process the "ALGOGram system") from morphine, duloxetine and indomethacin and the selective kappa-opioid receptor agonist U50 488H positive controls. Description of the animal models used are detailed at https://www.ans-biotech.com/in-vivo-models/.

An initial eight magnetic field signals (here called "cognates") were tested (Figure 3). A total of 10 different pain models were used in five different pain categories (Acute, Chronic, Neuropathic, Inflammatory and Visceral). These eight cognates were derived from fentanyl, hydromorphone, ketamine, cannabidiol (CBD), gabapentin, indomethacin, naproxen and dexamethasone. *ul*RFE® signals were delivered using EMulate's Voyager system, coupled to a wound copper coil designed to envelop a rat cage in an AC magnetic field (Figure 1). A White Noise magnetic field control and vehicle (sham control) were used for each pain model. All rats were exposed in pairs for 24 hours prior to testing. Assay measurements were accomplished 15 minutes after removal from the magnetic field.

A second, confirmatory study (Figures 4-9) was done with select signals and pain models, powered for statistical analysis (N=10 rats per signal and per model). Cognates for fentanyl, cannabidiol (CBD), hydromorphone, dexamethasone, naproxen and indomethacin were tested in rats, across five distinct pain models.

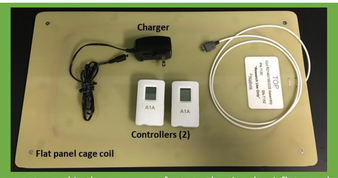

Figure 1 – Components used in the exposure of rats to the signals. A flat panel cage coil two controllers and a charger were the basic components delivered to ANS for use in our sponsored studies.

Figure 2 – Cages fit within the emitted field of the flat panel cage coil. Rats are exposed

In both screen and confirmatory assays, rats were exposed, full body, to the individual signals via proprietary emitter systems and flan panel coils (Figure 1). Two controllers per signal were used to deliver continuous exposure to rats in these cages. A standard plastic cage was used to house single or paired rats (dependent of the experiment) during exposure (Figure 2). Voyagers (the white Controllers) are powered via medical grade batteries and can last 14-16 hours. Voyagers were swapped every 12 hours to ensure continuous exposure. Voyagers were recharged, using the charger displayed in Figure 1.

Results: In April of 2021, a series of screens were run to test the potential pain reduction effects of magnetic field recordings of molecular compounds. These magnetic fields were recorded via our MIDS system [4] and loaded onto Voyager controllers. Fentanyl, hydromorphone, CBD, ketamine, naproxen, dexamethasone, indomethacin and white noise (Figure 1) were screened. Results were compared against historical controls of morphine, U50488H, indomethacin and duloxetine (Figure 1, grey area). The results are displayed in both a radar plot and in a table format (Table1).

In the screen CBD, fentanyl, hydromorphone, naproxen, indomethacin and dexamethasone exhibited effects in neuropathic, inflammatory, acute and visceral pain. These signals and their variations were selected for further testing in confirmatory assays using the TNBS, Carrageenan, Oxaliplatin, Bennett paw pressure and Acetic acid pain models.

As shown in Figure 4, signals of naproxen, fentanyl and CBD identified with the TxU suffix exhibited statistically significant pain reduction effects in the oxaliplatin pain models, almost equivalent to the physical control drug duloxetine. Signals without the suffix designation, white noise and signals delivered via commercial amplifiers did not achieve statistical significance.

As shown in Figure 5, only the CBD TxU signal and white noise exhibited a statistically significant effect in the Carrageenan inflammatory pain model. Both signals delivered approximately 41-43% of the pain reduction sensation of physical indomethacin .

A shown in Figure 6, the Fentanyl TxU and the white noise signal demonstrated statistically significant effects, compared to the saline injection. Both signals delivered the equivalent of the physical compound U50488H (kappa-opioid agonist).

The results from the Acetic Acid (Figure 7) and Bennet Paw pressure (Figure 8) did not reveal a statistically significant effects and very modest changes in the signals applied.

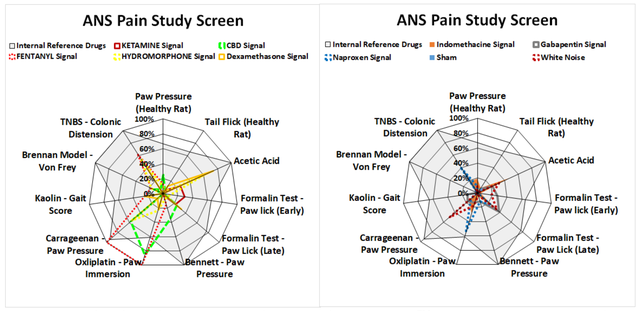

Figure 3 – Radar plots of relative pain reduction in the ALGOGram™ pain screen. Screens tested the effects of cognates derived from fentanyl, hydromorphone, CBD, ketamine, dexamethasone, indomethacin, gabapentin & naproxen.

Pain Model Tested	Internal Reference Drugs	KETAMINE COGNATE	CBD COGNATE	FENTANYL COGNATE	HYDROMORPHONE COGNATE	DEXAMETHASONE COGNATE	INDOMETHACINE COGNATE	GABAPENTIN COGNATE	NAPROXEN COGNATE	Sham	White Noise
Paw Pressure (Healthy Rat)	67%	0%	25%	13%	9%	0%	19%	0%	9%	0%	9%
Tail Flick (Healthy Rat)	66%	5%	5%	2%	3%	12%	8%	6%	0%	8%	0%
Acetic Acid	100%	25%	0%	26%	59%	74%	47%	22%	3%	11%	32%
Formalin Test - Paw lick (Early)	65%	29%	1%	0%	10%	0%	4%	27%	0%	0%	19%
Formalin Test - Paw Lick (Late)	77%	22%	24%	0%	7%	32%	31%	38%	25%	17%	34%
Bennett - Paw Pressure	100%	2%	35%	15%	8%	0%	5%	12%	12%	1%	4%
Oxliplatin - Paw Immersion	61%	3%	86%	100%	24%	22%	28%	13%	53%	0%	18%
Carrageenan - Paw Pressure	94%	5%	57%	100%	55%	10%	15%	17%	12%	1%	49%
Kaolin - Gait Score	65%	0%	0%	25%	0%	25%	0%	13%	0%	0%	0%
Brennan Model - Von Frey	100%	2%	13%	17%	17%	0%	0%	0%	0%	0%	0%
TNBS - Colonic Distension	100%	7%	7%	63%	55%	41%	18%	30%	41%	1%	2%

Table 1 – Percentage change from baseline in the ALGOGram™ pain screen. Values on the table represent the graphical values from the radar plot in Figure 3. Red filled rectangles were selected for further testing in the confirmatory testing.

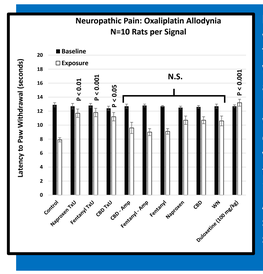

Figure 4 – The oxaliplatin neuropathic pain model of allodynia was used to test naproxen, fentanyl, CBD and white noise (WN). Two signals of Naproxen, Fentanyl & CBD were tested using the Voyager controller. Additionally, signals were delivered through a commercial MP3 amplifier (CBD – Amp & Fentanyl – Amp). Black columns represent the average baseline values for paw withdrawal (cold bath) for each of the pre-treatment and pre-exposure value groups. White columns represent the average values of post-oxaliplatin treatment and cognate exposure to the signals. Statistical analysis was compared to the pre induction baseline of the corresponding group, Wilcoxon test. As compared to the vehicle-treated group, Tuckey's test after significant Kruskal-Wallis ANOVA on ranks. Error bars are standard error of the mean (S.E.M.).

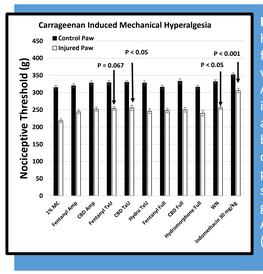

Figure 5 – The carrageenan pain model of mechanical hyperalgesia was used to test two signals of hydromorphone, fentanyl and CBD, delivered via the Voyager device. Signals were also delivered through a commercial MP3 amplifier (CBD – Amp & Fentanyl – Amp). A white noise (WN) signal was included as a magnetic field control with the signal delivered via a Voyager controller. Black columns represent the average baseline values for pressure applied to the foot pad in the control paw. White columns represent the average values of post-carrageenan treatment and cognate exposure to the signals. Statistical analysis was compared to the vehicle-treated group, Tuckey's test after significant Kruskal-Wallis One Way ANOVA on ranks. Error bars are standard error of the mean (S.E.M.).

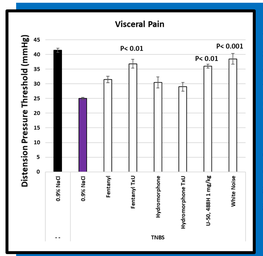

Figure 6 – The TNBS pain model of visceral pain was used to test two signals of fentanyl and hydromorphone.). A white noise signal was included as a magnetic field control with the signal delivered via a Voyager controller. The black column represent the average baseline distension values for balloon inflation (mmHg) in sham rats without surgical injury. The purple column represents the average distension applied. White columns represent the average values of post-surgical injuries and cognate exposure to the signals. Statistical analysis was compared to the pre induction baseline of the corresponding group, Wilcoxon test. As compared to the vehicle-treated group, Tuckey's test after significant Kruskal-Wallis ANOVA on ranks. Error bars are standard error of the mean (S.E.M.).

Conclusion/Discussion – The signals made from fentanyl, naproxen, CBD and white noise exhibited a statistically significant reduction in pain models. The consistency of the effect, with the exception of hydromorphone signals (Hydromorphone TxU & Hydromorphone) in the Visceral pain model, demonstrates that pain reduction was observed in specific signals selected for exposure. No adverse events were observed in any of the pain models, indicating a high safety profile for the *ul*RFE® system.

The replication and statistical confirmation of the observed screen effects in fentanyl, naproxen and CBD signals demonstrates that the effects are specific and real. The white noise signal results were significant, which suggests that a non-specific effect is present. This needs to be assessed with the knowledge that stochastic resonance effects do occur with pure white noise. Coupling to other cage coil signals may produce the observed effects with white noise. Additional tests, using signals from saline, should help us in identifying the effect.

Based on the results and safety profile observed in these assays, and previous experience in the oncology clinical trials with *ul*RFE®, we are ready to proceed with a feasibility (phase I) clinical trial.

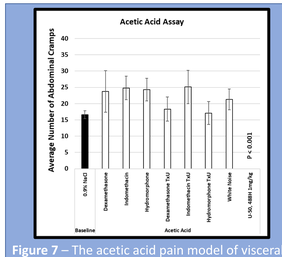

Figure 7 – The acetic acid pain model of visceral pain was used to test two signals of hydromorphone, dexamethasone and indomethacin. The physical compound U50488H, a kapa-opioid receptor agonist drug, was the only treatment group to show a statistically significant effect. All groups were analyzed relative to the baseline group using the Student's t-test (black column). Error bars are standard error of the mean (S.E.M.).

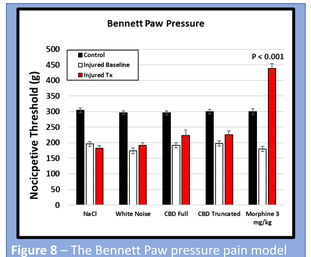

Figure 8 – The Bennett Paw pressure pain model of neuropathic nerve pain was used to test two CBD signals. The physical compound morphine, a mu-opioid receptor agonist drug, was the only treatment group to show a statistically significant effect. All groups were analyzed relative to the baseline group using the Student's t-test (black column). Error bars are standard error of the mean (S.E.M.).

References:

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