RESIDUAL ADRENAL FUNCTION IN AUTOIMMUNE ADDISON'S DISEASE – EFFECT OF DUAL THERAPY WITH RITUXIMAB AND DEPOT TETRACOSACTIDE

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ABSTRACT

CONTEXT In autoimmune Addison's disease (AAD), exogenous glucocorticoid (GC) therapy is an imperfect substitute for physiological GC secretion. Patients on long-term steroid replacement have increased morbidity, reduced life expectancy and poorer quality of life.

OBJECTIVE To restore adrenocortical steroidogenic function in recent onset AAD.

DESIGN Open-label, multi-centre trial of immunotherapy and trophic stimulation in new-onset AAD. Serial measurement of serum and urine corticosteroids at baseline and throughout 72-week follow-up period.

SETTING Endocrine Departments and Clinical Research Facilities at 5 UK tertiary centres.

PATIENTS Thirteen subjects (9 female, 4 male; aged 19-64 years) with AAD confirmed by high ACTH, low circulating cortisol (basal <100nmol/L or post-tetracosactide <300nmol/L) and positive serum 21-hydroxylase antibodies.

INTERVENTION All subjects received dual therapy with B-lymphocyte depleting immunotherapy (rituximab 1g given twice) and repeated depot tetracosactide (1mg alternate days for 12 weeks).

MAIN OUTCOME MEASURE Restoration of normal glucocorticoid secretion (stimulated cortisol >550nmol/L) at Week 48.

RESULTS Ten of 13 (77%) had detectable stimulated serum cortisol (26-265nmol/L) at trial entry. Following intervention, 7/13 (54%) had an increase in stimulated cortisol measurement, with a peak response of 325nmol/L at Week 18 in one subject. Increased steroid metabolites, assayed by urine GC-MS at Week 12 and Week 48, was detected in 8/13 (62%), reflecting an increase in endogenous steroidogenesis. Four of 13 had Residual Adrenal Function at 72 weeks.

CONCLUSION Combined treatment with rituximab and depot tetracosactide did not restore normal adrenal function. Nevertheless, adrenocortical plasticity is demonstrated in some patients and this has the potential to be exploited to improve adrenal function.

Clinical Trial registration (ISRCTN) 20220821

Introduction

Autoimmune Addison's disease (AAD) is a rare disease in which immune-mediated destruction of steroid-producing cells in the adrenal cortex culminates in a potentially fatal state of steroid deficiency (1,2). Steroid 21-hydroxylase and other adrenal steroidogenic enzymes are the target of immunological attack (3). Once levels of circulating glucocorticoids (GC) and mineralocorticoids (MC) fall to a critical state, patients are absolutely dependent on daily steroid replacement for survival.

The advent of cortisone acetate in the 1940s transformed the disease from certainly fatal to a manageable chronic condition. Nonetheless, synthetic GCs cannot mimic the intrinsic diurnal rhythm of cortisol production (4), thus current steroid replacement regimens are imperfect. Side-effects from even a subtle excess of glucocorticoid pose a risk to bone health, cardiovascular risk and glucose tolerance (5-10). Despite regular steroid replacement, the risk of adrenal crisis remains an unpredictable and dangerous threat to health, and life expectancy is reduced in patients with AAD (11-13).

Adrenocortical plasticity has long been established (14), with several examples in clinical practice: patients receiving exogenous steroid therapy develop adrenal atrophy and functional adrenal failure; conversely, hypertrophy of the adrenal glands is seen in the setting of ACTH excess (e.g. Cushing's disease). Recent early-phase studies of novel therapies have significantly advanced our understanding of the concept of Residual Adrenal Function (RAF) in AAD, and have suggested that adrenocortical plasticity may be amenable to intervention (15,16). The use of B cell-depleting immunotherapy in autoimmune disorders that share pathophysiological features with AAD has now translated to routine clinical care for some but not all conditions (17-19), and the first study in AAD treated 6 newly diagnosed patients with rituximab with some success (15). This B-lymphocyte depleting anti-CD20

immunotherapy ameliorated the immunological destruction of steroid-producing cells in the adrenal gland in one patient - progressively rising concentrations of endogenous GCs and MCs were seen, allowing a temporary complete cessation of replacement steroids (15). Thereafter, a second study of regenerative therapy in AAD was performed: 13 patients with established AAD of greater than 1 year duration were treated with repeated doses of tetracosactide ($ACTH_{1-24}$, depot Synacthen). This trophic stimulation harnessed and exploited RAF in two patients (4 and 8 years from diagnosis) - levels of intrinsic GCs and MCs rose, and both patients stopped exogenous steroids entirely (16). One patient remains off steroid replacement seven years later.

These early-phase studies have greatly enhanced our understanding of the potential of RAF and the impact regenerative medicine therapy could have in AAD. This paper reports the RADS2 study, which combined therapy with B-lymphocyte depleting immunotherapy and trophic ACTH stimulation in newly diagnosed patients for the first time, with the aim of harnessing and exploiting endogenous adrenal steroidogenesis and ultimately delivering better outcomes for patients with this chronic disease.

Patients and Methods

Thirteen subjects (9 female, 4 male; aged 19-64 years), with a diagnosis of new onset AAD within the preceding four weeks were recruited from endocrine or acute medical services in Newcastle, Exeter, Cambridge or Cardiff, UK. Patients underwent robust clinical and biochemical screening at the point of trial entry to confirm unequivocally that adrenal failure was primary and of autoimmune origin. Eligibility criteria included: age 10-65 years, clinical features to confirm primary adrenal failure, high ACTH (>47 ng/L), low circulating cortisol concentrations (basal <100nmol/L or stimulated 30 or 60 minutes post-tetracosactide <300nmol/L) and positive serum 21-hydroxylase antibodies (≥1U/mL). A computed

tomography scan and chest x-ray were also performed to exclude intercurrent illness or malignancy and to assess adrenal gland appearances. Exclusion criteria were: significant cardiovascular or respiratory disease (including asthma), renal or hepatic disease, malignancy, pregnancy or breastfeeding, current infectious disease (including HIV, hepatitis B/C, shingles/zoster, tuberculosis), unexplained abnormality on chest x-ray and previous use of immunosuppressive or cytotoxic drugs (excluding GC).

33 potential recruits were identified and underwent preliminary screening across 4 sites. 17 patients were consented and formally recruited into the 72-week study (**Figure 1**). Of those consented, 4/17 patients failed one or more eligibility criteria for treatment (**Figure 2**).

The study was registered at ISRCTN with ID 20220821. Ethical approval was granted by the National Research Ethics Service North East-Sunderland, reference 12/NE/0339.

Design and Intervention Regimen

This open-label study of rituximab and depot tetracosactide followed newly diagnosed patients for 72 weeks after intervention to assess for any improvement in adrenocortical function. The schedule of visits for screening, intervention and follow-up is outlined in **Figure 1**.

Rituximab (1 gram by intravenous infusion) was administered on Day 1 and Day 15 of the study. Patients were taught to self-inject 1mg subcutaneous depot tetracosactide on Day 1 and this was administered on alternate days for a minimum period of 12 weeks. In participants who had any biochemical evidence of a rising stimulated-cortisol, tetracosactide was continued for a maximum period of 20 weeks in total.

At recruitment, all participants were taking hydrocortisone as GC replacement (in doses ranging between a total of 15-50mg daily). A subset of patients had not yet commenced fludrocortisone, so this was started at the first clinical encounter with the trial team. GC replacement doses were lowered to a total daily dose of 10-15mg hydrocortisone where possible, to promote maximal endogenous ACTH secretion. Throughout the study, in participants with a measurable improvement in endogenous steroidogenesis (any rise in basal or stimulated-cortisol concentrations), GC replacement was judiciously weaned, with regular monitoring of clinical symptoms, blood pressure and serum electrolytes. The lowest daily GC replacement dose reached was 5mg hydrocortisone daily in one patient.

Outcome Measures and Assessments

The primary outcome measure was restoration of normal GC secretion at Week 48, defined as a peak stimulated cortisol of >550nmol/L. Secondary outcome measures were: restoration of normal GC secretion at Weeks 6, 12, 24 and 72, improvement of basal or peak cortisol (>100nmol/L over baseline), changes of other biochemical parameters (DHEA-S, 17OHP) and the safety and tolerability of the regimen.

Participants had regular follow-up during the first 24 weeks of the study, with clinical assessment +/- biochemical assessment on Day 1, 7, 14, 28 and then at 6, 12, 18 and 24 weeks. Major outcome visits included a SST and detailed serum biochemistry sampling and were performed at Week 6, 12, 24, 48 and 72, during a 36-hour 'steroid medication-free' window to allow assessment of endogenous steroid production (**Figure 1**). Overnight urine collections were performed during the steroid-free window and a comprehensive panel of urine steroids were measured at Baseline, Week 12 and Week 48. Participants underwent robust education and overnight hospital admission while steroid-free to ensure safety. In the event of intercurrent illness, the visit was postponed for a short time; in one instance, a visit

was cancelled. Electrolytes, full blood count, lymphocyte subsets and short synacthen tests (SST, off replacement steroids) were analysed in real-time. All other blood samples and urine collections were stored at -80°C, and batch analysed on trial completion.

Flow cytometry analysis of B-lymphocyte subsets was performed on fresh material throughout the study to assess the depth of B-lymphocyte depletion. CD19+ cells were measured at baseline and following intervention; 10,000 lymphocyte events were counted twice at each measurement. Complete depletion was judged as CD19+ <0.1% of lymphocytes.

Short synacthen tests (SST with cortisol measured by competitive chemoluminescent assay, lower limit of detection (LLD) 24nmol/L) were performed at Baseline, 6, 12, 24, 48 and 72 weeks and processed centrally with analysis performed in real time. 250µg soluble Synacthen ($ACTH_{1-24}$) was administered intramuscularly following a baseline blood sample drawn for cortisol measurement, with further samples drawn at 30 and 60 minutes. Prior to SST, a series of serum and plasma samples were drawn and stored to allow batch analysis of ACTH (solid-phase, chemoluminescent assay, LLD=5ng/L), dehydroepiandrosterone sulfate (DHEA-S; solid-phase competitive chemoluminescent assay, LLD=0.1umol/L), androstenedione (solid-phase competitive chemoluminescent assay, LLD=1.05nmol/L), aldosterone (solid-phase radioimmunoassay, LLD=70pmol/L), 17-hydroxyprogesterone levels (17OHP; radioimmunoassay, LLD=1nmol/L) and 21OH Abs (ELISA kit from RSR Ltd (Cardiff); positive result ≥1.0U/mL) (20).

A comprehensive panel of urine steroids, collected in the steroid medication-free window, were measured at Baseline, Week 12 and Week 48: GC precursors, GC metabolites, MC precursors, MC metabolites and androgens were measured by gas chromatography-mass

spectrometry (GC-MS) in the laboratory at the Steroid Metabolome Analysis Core, Institute of Metabolism and Systems Research, Birmingham. 32 individual urinary steroids was quantified on an Agilent 5975 instrument after free and conjugated steroids were extracted from 1ml of urine by solid-phase extraction (21). Urine metabolomic results were corrected for collection duration. No female patients were taking the oral contraceptive pill or hormone replacement therapy during the urine sample collections.

One major outcome visit was missed entirely due to illness (unsafe to stop steroid replacement medication) and other safety visits were delayed by several days because of unavoidable commitments that participants could not reschedule. This was an uncontrolled exploratory study and descriptive statistics are used to present outcome measurements. Where appropriate, continuous variables were analysed by paired t-tests.

Results

Participant baseline characteristics

Twelve of 13 participants (mean age 44; range 19-64 years) reported they had experienced weight loss, nausea or vomiting and postural symptoms prior to diagnosis. Eleven of 13 reported salt craving, and all 13 described fatigue or lethargy. Eleven of 13 were pigmented and 9/13 were in 'crisis' at the point of diagnosis (with adrenal crisis defined as requiring hospital admission for parental steroids and intravenous fluids). 7/13 had concurrent autoimmune diseases (hypothyroidism n=5, pernicious anaemia n=2, Graves' disease n=1 and premature ovarian failure n=1), with one participant having a triad of autoimmune hypothyroidism, pernicious anaemia and premature ovarian failure (**Table 1**).

Ten of 13 participants had detectable but subnormal stimulated cortisol on SST at formal screening at trial entry (26-265nmol/L). All had elevated ACTH levels (68-2630ng/L; NR 0-47ng/L) and positive 21OH Abs (2.8-3648U/mL; NR<1U/mL)(**Table 1**).

Adrenal steroidogenic function: Serum

Seven of 13 participants demonstrated a rise in endogenous cortisol following intervention (an increase in stimulated cortisol on SST detectable during sampling at least one major outcome visit; *P*=0.45 at Week 48 *vs* Baseline visit; paired t-test)(**Figure 3**). No participants met the primary study outcome with restoration of normal endogenous steroidogenesis (stimulated cortisol >550nmol/L), but one participant (Participant 5) did achieve a secondary outcome measure with an increase in stimulated cortisol from 55nmol/L to 155nmol/L following intervention. This female patient (aged 24) had clear evidence of sustained endogenous steroidogenesis following rituximab therapy and adrenocortical stimulation. Of note, this patient had the highest titre of 21-hydroxylase antibodies (21OH Abs) at trial entry (3648U/mL) and the longest duration of symptoms prior to diagnosis (fatigue and hyperpigmentation of several years duration).

Participant 4 had the highest recorded serum cortisol during the study – 284nmol/L post-synacthen at Week 12, (an early morning cortisol measurement taken as part of safety surveillance at Week 18 was 325nmol/L). This 56 year old female participant retained clear evidence of endogenous steroidogenesis for over 12 months after trial entry.

Participant 10 did not meet any biochemical endpoints, but did have noteworthy endogenous function throughout the study. At trial entry, his peak cortisol was 145nmol/L, with a significant rise to a peak cortisol of 234nmol/L at Week 6. Depot tetracosactide was continued for 20 weeks - he retained detectable endogenous steroidogenesis at Week 72

(peak cortisol 114nmol/L on stimulation). A further male patient (Participant 13) maintained endogenous steroidogenesis throughout the 72-week follow-up period with a stimulated cortisol at trial entry of 81nmol/L, 127nmol/L at Week 48 and 116nmol/L at Week 72. At week 72, 4 of the 13 (31%) participants had stimulated serum cortisol concentrations of 99nmol/l or above, suggesting residual adrenal function. These four participants had higher mean serum cortisol at baseline than the rest of the cohort (129nmol/l vs 41nmol/l; p=0.03) but were not different with regard other baseline characteristics.

Measurements of DHEA-S, androstenedione, aldosterone and 17OHP in serum are shown in **Figure 4**. Aldosterone was undetectable throughout in all patients, except at Baseline and at Week 12 in Participant 4, who had the highest recorded stimulated cortisol in study. 17OHP was higher in female subjects, reflecting the contribution of an ovarian source. Similarly, serum DHEA-S concentrations were higher in men.

Adrenal steroidogenic function: Urine

Eight of 13 participants demonstrated rising urinary steroid metabolite excretion post-intervention, indicating an increase in endogenous adrenal steroidogenesis. In these eight participants, we saw a pattern of increased GC precursor excretion, notably 17-hydroxypregnanolone, pregnanetriol and tetrahydro-11-deoxycortisol, during the first 12 weeks of the study in several participants, with a subsequent decline by Week 48. Total GC metabolite production followed a similar pattern in a smaller numbers of participants (4/13), with an increase in urinary GC excretion between Baseline and Week 12 (**Figure 5**). Three participants (4, 10 and 13) who maintained a peak serum cortisol >100nmol/L at Week 72 had excreted the highest amounts of GC metabolites amongst all participants (**Figure 5**). A fourth individual (participant 01) had significant changes in urinary steroid output, with

increases in a range of GC precursors (pregnanediol, 17hydroxy-pregnanolone, pregnanetriol, pregnanetriolone and tetrahydro-11-deoxycortisol). This increase in production of steroid precursors indicates an authentic steroidogenic response to ACTH stimulation. Four of 13 participants had an increase in total MC metabolite production from Baseline to Week 12 and 5 of 13 participants had an increase androgen metabolite production during the same period when comparing pre *vs.* post-intervention. Pooling results from all the participants, there was no statistically significant increase in excretion of urine steroid metabolites between Baseline *vs*. Week 48.

In terms of correlation between serum and urine steroid response, 7 of 13 participants had a detectable serum response (any rise in stimulated cortisol from baseline), whereas 8 of 13 participants had a detectable rise in urine steroid excretion (overlapping with 6 of the serum responders). Notably, the number and range of increasing urinary steroid metabolites excreted post-treatment is not necessarily reflected in the serum steroid response. For example, Participant 01 had only a small increase in peak stimulated cortisol following intervention (increment of 30nmol/L in stimulated cortisol measurement at Week 12), but demonstrated increased urinary steroid metabolite excretion across the spectrum including GCs, GC precursors, MCs, androgens and androgen precursors.

Immune parameters

At baseline, 21OH Ab titres ranged from 2.8-3648U/mL (positive result ≥1.0U/mL). Serum immunoglobulin M levels fell over the course of the study, but remained within reference range (supplementary figure; reference 22). Twelve of 13 participants achieved CD19+ counts measured as 0.0 or 0.1% of the lymphocyte population following immunotherapy, with counts remaining low for several months (minimum of 12 – maximum of 48 depleted weeks). Resurgence (>0.5% of lymphocyte population) was detectable in all participants by

the end of the study, and occurred after a median period of 48 weeks (range 12-72 weeks) (supplementary figure)(22). Participant 08 did not achieve complete CD19+ depletion (lowest count 0.2% at week 6), and did not have a rise in stimulated cortisol following intervention.

Safety and tolerability

Trial medications were well tolerated by all participants. All infusions of rituximab were completed. All patients completed the active treatment phase; 1/13 did not complete the 72 week follow-up period (attended until Week 48, did not attend final Week 72 visit). Localised reactions to tetracosactide were frequently reported – redness, swelling and bruising around abdominal injection sites (these reactions have been previously reported with repeated doses of tetracosactide (16,23).

Four serious adverse events (SAEs) were recorded during the study; none were found to be causally-related to the study interventions (listed in supplementary table) (22). Multiple adverse events (AEs) were recorded across all sites during the study; frequently reported symptoms or minor illnesses were headaches, back pain, sore throat and sinusitis.

Discussion

Recent early-phase experimental studies of novel therapies have enabled small numbers of patients with AAD to wean and stop steroid replacement following intervention, transforming a chronic disease to a potentially curable condition (15,16, reviewed 24): this heralds an opportunity for a transformation in AAD management. The aim of this study was to combine immunotherapy and trophic stimulation to harness residual adrenal function (RAF), aiming to regenerate steroidogenic function, with the hope that dependence on steroid replacement and patient outcomes could improve further with dual therapy. It was anticipated

that rises in serum and urine glucocorticoids post-intervention would reflect improving endogenous steroidogenesis, with these biochemical changes potentially mirrored by enhanced QoL indicators and fewer adrenal crises – ultimately less morbidity and a reduced disease burden for patients.

No participants met the primary study outcome of restoration of endogenous steroidogenesis demonstrated by a stimulated cortisol >550nmol/L; nevertheless, 54% (7/13) did demonstrate a rise in serum cortisol post-intervention. The highest serum cortisol mid-study was achieved by Participant 04 (stimulated cortisol was 284nmol/L at Week 12): this allowed GC replacement to be weaned to 5mg hydrocortisone/day for 5 months. Participant 05 achieved a secondary outcome measure with a rise in serum cortisol of 100nmol/L, and 4 others (04, 06, 10 and 13) had sound biochemical evidence of maintained RAF, manifest as serum cortisol of 99nmol/l or more at 72-week follow-up. Although pathophysiologically interesting, this residual steroidogenic function is not of the magnitude required to make a difference to the clinical wellbeing or hormone replacement of these patients. Furthermore, as there was no control group, we cannot exclude that the low-level persisting adrenal function was owing to the spontaneous natural history of the condition and unrelated to the trial medication.

Urine GC/MS did herald meaningful results with 62% (8/13) demonstrating a rising excretion of urinary steroid metabolites post-intervention. This pattern of urinary steroid excretion was variable between participants, but one (participant 01), had a demonstrable increase across panels of glucocorticoids metabolites, glucocorticoid precursors and androgens. Importantly, the increase in steroid precursor metabolites unequivocally indicates improving endogenous adrenocortical function. While the numbers of participants in this study, and therefore those with a detectable positive response, are low, this is objective evidence of improving RAF many months after a proven diagnosis of AAD. In the previous

study using $ACTH_{1-24}$ stimulation (16), analysis of urine steroid profiles in the 2 participants who responded revealed that urinary excretion of GC precursors and active GC metabolites gradually increased from below the 5th centile to above the median of healthy female controls at 10 weeks (in one responder) and at 40 weeks (in second responder), with a parallel increase in urine MC metabolite excretion. In both patients, androgen precursor and active androgen metabolite excretion was slower to rise.

In the current study, when urine response is compared to levels of steroids detected in serum, it is apparent that serum steroid measurements cannot provide a comprehensive illustration of endogenous gland function. Therefore, assessment of urinary steroid excretion should be considered the most reliable method of analysis of endogenous steroidogenesis in future studies: urine steroid assays are our most valuable and robust tool for appraising adrenal gland function.

Around 40% of patients with AAD have never been hospitalised with an adrenal crisis (25, 26); perhaps a reduced risk of crisis might be correlated to detectable RAF which will have a protective effect. The two participants who responded to ACTH stimulation in the previous early phase study had never been hospitalised with an adrenal crisis (16). In the current study, Participant 04 presented with a crisis (defined by hospital admission with requirement for parenteral steroids and intravenous fluids), but Participant 05 did not. Clearly there is insufficient data in this study to predict how protective RAF may be, but it does warrant further exploration because emerging data suggest it is not rare amongst patients with established AAD (16,27,28). Furthermore, RAF has the potential to positively impact the risk of life-threatening crisis, alongside other morbidity and mortality factors.

In healthy individuals, pulsatile ACTH secretion from the pituitary starts around 0300h and increases to a zenith around 0700h, accompanied by a concordant but delayed rise in adrenal cortisol secretion. As the day progresses, ACTH pulse amplitude decreases, leading to reduced levels of serum cortisol by the late afternoon and evening: this circadian variation is the key to optimising GC replacement in patients with Addison's disease (4,29). Exogenous steroid replacement cannot replicate this entirely, which is one of the key drivers for continuing to investigate methods of harnessing and exploiting endogenous steroidogenic capability. The presence of RAF and the fluctuating nature of endogenous steroidogenesis observed in this and previous studies reflects the heterogeneity of AAD. Previously, it was a widespread assumption that the instigation of exogenous steroids caused adrenal glands to entirely cease to function. It seems probable that ACTH drive diminishes rapidly following the start of steroid replacement in most patients, compounding functional steroidogenic failure. However, these studies have accumulated evidence that intrinsic gland function can exist, even years after diagnosis, and has the potential to be exploited. Discovery of this heterogeneity is comparable to gains in knowledge in recent years of a spectrum of disease and a subset of patients with persisting C-peptide positivity indicative of a degree of maintained ß-cell function in type 1 diabetes. In both conditions, the disease trajectory within and between individuals is variable, resulting in greater scope for intervention to harness residual gland function and potentially improve patient outcomes – a further example of opportunity for the development of personalised medicine.

One significant challenge in detecting and monitoring RAF is the requirement for daily steroids in AAD. This limits the assessment of intrinsic gland function in a routine, outpatient clinical setting. The identification of a biomarker which could act as a surrogate for endogenous glucocorticoid production would be particularly advantageous. Further attention should also be given to the choice of immunotherapy administered as rescue therapy

in the setting of immune-mediated adrenocortical destruction. Rituximab was chosen in this early-phase study because of its mechanism of action, efficacy in similar diseases, partially successful outcome in our initial study (15) and its durable safety record over two decades. While some participants experienced mild side-effects during administration of the drug, it was essentially well-tolerated – a key consideration in an experimental study utilising novel therapeutic approach. Alternative immunotherapies, such as those which can maintain or enhance the activity of regulatory T-cells, may prove more robust for tackling diseases with immune-mediated gland destruction, such as type 1 diabetes (30) and AAD, but are likely to be less well tolerated. Consequently, they may not be acceptable to patients considering participation in early-phase studies or panels considering ethical approval. Furthermore, it is evident from this study that once repeated ACTH stimulation is withdrawn, its effect on adrenocortical steroid production rapidly wanes in most cases.

Conclusion

While harnessing and exploiting RAF remains a significant challenge, this experimental study has added further weight to the evidence that a sizeable proportion of patients with AAD have maintained endogenous steroidogenic potential after diagnosis. Our understanding of physiological steroid production means we know that standard steroid replacement is an imperfect therapy for patients with AAD – but what does detectable RAF or an improvement in endogenous function really mean for patients? There is a wealth of evidence that patients with the condition exhibit increased morbidity and reduced quality of life: improved intrinsic gland function can be expected to counteract these problems to a degree, although the numbers in pilot clinical studies are too small to provide robust evidence of superior outcomes in the context of improving RAF. Nevertheless, there have been no trials of novel therapies other than alternative steroid replacement for AAD for over half a century:

persisting with innovative therapeutic approaches is the only meaningful prospect for delivering a tangible improvement in the lives of those with the condition.

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Table 1. Clinical and Biochemical Characteristics at Baseline

PARTICIPANT	AGE	SEX	BASELINE STIMULATED CORTISOL (nmol/L)*	ACTH (ng/L) AT STUDY ENTRY	21OH ANTIBODIES (U/mL) AT STUDY ENTRY	OTHER AUTOIMMUNE DISEASE
1	45	F	40	1085	6.0	
2	64	F	<24	68	22.3	GD
3	36	F	<24	1050	39.6	PA
4	**56**	**F**	265	316	5.5	AH
5	24	F	55	827	3648	
6	**56**	**F**	26	915	11.6	AH
7	43	F	<24	850	71.7	AH
8	27	M	30	1054	413.3	
9	19	M	40	1542	581.7	
10	**48**	**M**	145	535	10.8	
11	60	F	45	1160	63.4	AH, PA, POF
12	39	F	88	393	17.0	AH
13	**52**	**M**	81	2630	2.8	

13 treated participants. Mean age 44 years (range 19-64). Stimulated cortisol on Short Synacthen Test (SST) at study entry ranges from <24 to 265nmol/L; the peak value of 30 or 60 minutes post-tetracosactide is shown. Demographic features of those who had serum cortisol ≥99nmol/l at 72 weeks are highlighted in bold. *To convert serum cortisol values to μg/dl divide by 27.6. ACTH at baseline ranged between 68-2630ng/L (NR 0-47ng/L) and all 12 participants had positive 21OH antibodies (2.8-3648U/mL; NR<1U/mL).

GD = Graves' disease. PA = pernicious anaemia. AH = autoimmune hypothyroidism.

Figure Legends

Figure 1. Screening, Treatment and Monitoring Schedule in the RADS2 Study. Initial screening was performed in-person, or by telephone or email. If this identified a patient with primary adrenal failure who was willing to participate in the study, then formal eligibility testing took place. Robust clinical and biochemical assessment was carried out at the study entry (Baseline visit), prior to any intervention. Major outcome visits (shown in middle column) were performed with patients 'free' of exogenous steroids, allowing assessment of endogenous steroid production. Interim safety visits (shown in right hand column) allowed a shorter clinical assessment to take place, with the primary aim being patient safety.
*depot tetracosactide therapy started. 1mg administered subcutaneously on alternate days
**depot tetracosactide discontinued at Week 12, or continued to a maximum of Week 20, depending upon response (rising stimulated cortisol on Week 6 and Week 12 SST) and tolerability

Figure 2. 'CONSORT' flow diagram of Patients Screened, Enrolled and Treated in the RADS2 Study. 33 patients with adrenal failure diagnosed within the past 4 weeks were contacted by a member of the study team, for discussion of the study and initial assessment of clinical history. 17/33 patients with primary adrenal failure who agreed to participate proceeded to formal consent and recruitment into the study. These patients were then formally screened to ensure they unquestionably met eligibility criteria. 4 patients did not meet eligibility criteria on comprehensive assessment and could not proceed. 13 participants received treatment with rituximab and depot tetracosactide. 12 participants completed 72 weeks of follow-up.

Figure 3. Peak Stimulated Cortisol on Short Synacthen testing at Baseline and Major Outcome Visits. Peak stimulated cortisol (higher value of 30 or 60 minutes post-tetracosactide) at Baseline (study entry) and at each major outcome visit: Week 6, 12, 24, 48 (primary outcome assessment) and 72. Seven of 13 participants had an increase in stimulated cortisol recorded on ≥1 follow-up visit. 4 participants (04, 06, 10 and 13) completed the study with a stimulated cortisol measurement of ≥ 99nmol/L. Male participants are shown in blue. Lower limit detection/LLD=24nmol/L.

Figure 4. Serum Steroid Biochemistry at Baseline and Major Outcome Visits. DHEA-S (μmol/L; solid-phase competitive chemoluminescent assay, lower limit detection/LLD=0.1μmol/L), 17aOHP (nmol/L; radioimmunoassay, LLD=1nmol/L), aldosterone (pmol/L; solid-phase radioimmunoassay, LLD=70pmol/) and androstenedione (nmol/L; solid-phase competitive chemoluminescent assay, LLD=1.05nmol/L) were measured in all treated participants at each major outcome assessment (Baseline, Week 6, Week 12, Week 24, Week 48 and Week 72). Samples were collected prior to each major outcome visit SST and batch analysis was performed on trial completion. Grey shading denotes lower limit detection/LLD for each steroid measured. Male participants are shown in blue. Participants with the highest levels of DHEA-S are all male, likely representing a testicular source of DHEA-S. No patients were taking DHEA supplementation.

Figure 5. Urine Steroid Metabolite Excretion (µg/24 hours) at Baseline, Week 12 and Week 48

A comprehensive urine steroid profile was measured by gas chromatography-mass spectrometry at Baseline, Week 12 and Week 48 (primary outcome assessment) of the study (see methods section; 21). The sum of the metabolites of the glucocorticoid precursors (17-hydroxyprogesterone, 17-hydroxy-pregnanolone, pregnanetriol) and 11-deoxycortisol (tetrahydro-11-deoxycortisol) are plotted (panel A). The sum of the active glucocorticoid metabolites (cortisol, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortol, β-cortol, cortisone, tetrahydrocortisone, α-cortolone, and β-cortolone) are shown (panel B). The sum of the mineralocorticoid metabolites (3α,5β-tetrahydroaldosterone, tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, tetrahydrodeoxycorticosterone, 5α-tetrahydrodeoxycorticosterone, tetrahydro-11-dehydrocorticosterone, and 5α-tetrahydro-11-dehydrocorticosterone) are plotted (panel C). The sum of the androgen precursor metabolites dehydroepiandrosterone, 16α-dehydroepiandrosterone, 5-pregnanediol and 5-pregnanetriol are plotted (panel D). The sum of the major active androgen metabolites androsterone and etiocholanolone are plotted over time (panel E). Total urine GC metabolite production (shown here; estimated production in µg/24hours) increases between study entry and Week 12 in 4/13 participants (01, 05, 12, and 13). Three of 13 participants (04, 10 and 13) with stimulated cortisol >100nmol/L at Week 72 had the highest levels of GC metabolite production in urine. Baseline urine samples from participant 8 and participant 10 were not available.