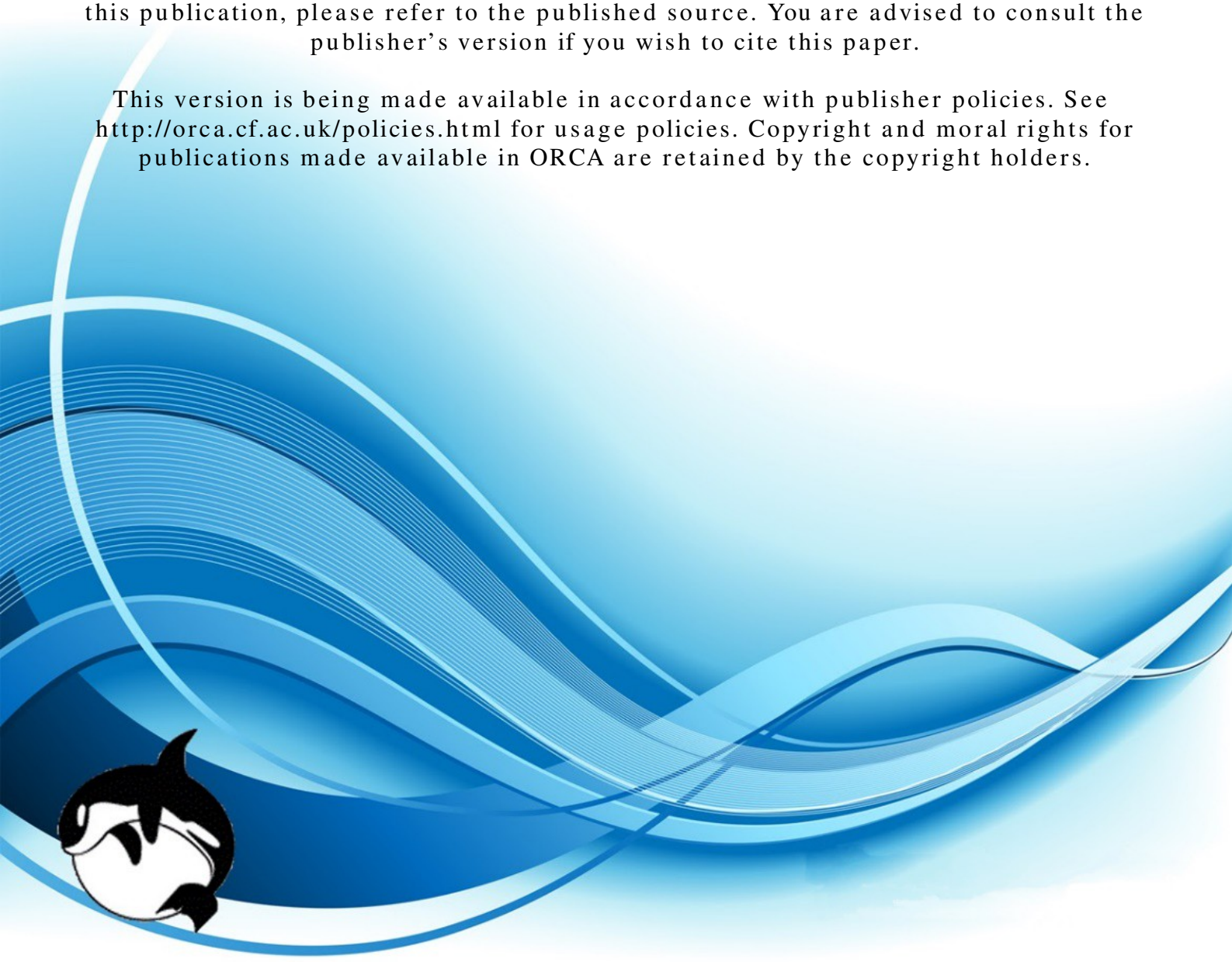

Designing a combined Liothyronine (LT3), L-thyroxine (LT4) trial in symptomatic hypothyroid subjects on LT4 – the importance of patient selection, choice of LT3 and trial design

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Abstract

10-15% of subjects with hypothyroidism on L-thyroxine (LT4) alone, have persistent symptoms affecting their quality of life (QoL). Although the cause for this is unclear, there is evidence that "tissue T3 lack" may be responsible. If so, combining liothyronine (LT3) with LT4 should help.

However, randomised controlled trials (RCT), have not established greater efficacy for the LT3+LT4 combination in these subjects compared to LT4 alone. While trial design may have been responsible, the use of unphysiological, short acting LT3 preparations and non-thyroid specific patient reported outcome measures (PROMs) may have contributed.

We recommend attention to the following aspects of trial design for future RCTs of LT3+LT4 compared to LT4 alone –

(a) Subject selection – (i) measurable symptoms (disadvantages should be recognised); (ii) using a validated thyroid specific PROM such as ThyPRO39 or the Composite scale derived from it; (iii) those taking over 1.2μg/day or 100μg/day (for pragmatic reasons) of LT4 defining a population likely without intrinsic thyroid activity who depend on exogenous LT4; (iv) recruiting a preponderance of subjects with autoimmune thyroiditis increasing generalisability; and (v) those with a high symptom load with a greater response to combination therapy e.g. those with the deiodinase 2 polymorphism.
(b) The use of physiological LT3 preparations producing pharmacokinetic similarities to T3 profiles in unaffected subjects – 2 long acting LT3 preparations are currently available which have to be tested in phase 2b/3 RCTs.
(c) The superiority of a crossover design in limiting numbers and costs while maintaining statistical power and ensuring all subjects experience the investigative medication.

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Introduction

Eighteen randomised controlled trials (RCT) of combined liothyronine (LT3) and L-thyroxine (LT4) therapy (LT3+LT4) have been inconclusive about its superiority in improving symptoms and quality of life (QoL) in hypothyroid subjects dissatisfied with LT4 alone (1) (Table 1). While 16 RCTs used short acting LT3 preparations in patients with primary hypothyroidism (autoimmune, post-radioiodine, post-thyroidectomy), one used natural desiccated thyroid (NDT) and one investigated subjects with central hypothyroidism (2,3). This group of "dissatisfied" hypothyroid subjects on LT4 monotherapy constitutes a significant number estimated to be 10-15% of all hypothyroid patients in the UK (4-6). The unmet need is therefore great. Their number will increase because of (a) the rising prevalence of hypothyroidism partly accounted for by lower thresholds for starting LT4 in an ageing population (7,8) – prevalence in the USA increased from 9.5% (2012) to 11.7% (2019) with over 78% receiving LT4 alone (9); and (b) the significant proportion of Endocrinologists (51%) who are willing to consider prescribing LT3 for these patients (10).

These patients have many symptoms (fatigue, low energy and mood, memory deficits, "brain fog") and physiological abnormalities [high BMI, abnormal body composition, low resting energy expenditure (REE)] which significantly affect QoL. Hypothyroid subjects on long-term LT4 alone have: (a) a 15% excess of psychological caseness (5), a 5% increase in BMI, raised cholesterol and a 25-30% increase in cardiovascular and all-cause mortality (10-13); and (b) unphysiological T4/T3 ratios with higher T4 than in the normal population (12,14). They have many futile consultations and investigations leading to dissatisfaction with their hypothyroidism management. Some of these investigations pertain to excluding coexisting other causes for their symptoms and should be pursued.

The possible causes for persistent symptoms in these subjects have been reviewed elsewhere (15,16). Of these, there is now evidence from animal experiments, population studies, and human experiments to support the "low tissue T3" hypothesis in some (17,18). It is plausible that this is caused by exogenous LT4 induced inhibition of deiodinase 2 expression (D2, the main enzyme converting intracellular "prohormone" T4 to "active" T3) with differential effects on D2 activity in hypothalamo-pituitary tissues (D2 largely unaffected by excess T4) and peripheral tissues (D2 expression suppressed)(19). These differences in D2 activity normalise serum TSH (the "gold standard" for LT4 dosing), while peripheral tissues remain unreplenished with T3 converted from the same LT4. It would therefore seem logical to give them LT3 combined with LT4 since "tissue T3 lack" may account for their persistent symptoms and poor QoL.

Designing a RCT in hypothyroid subjects on LT4 with persistent symptoms

The primary objective of a high quality blinded RCT investigating LT3+LT4 therapy in hypothyroidism is to provide phase 2b efficacy data that LT3+LT4 is superior to LT4 alone in improving persistent symptoms and QoL while restoring physiological thyroid hormone profiles. The use of patient reported outcome measures (PROMs) as primary outcome in RCTs is now internationally validated and accepted (20,21). Data should also be sought on pharmacokinetics, safety and secondary outcomes [weight/BMI, body composition, REE, metabolomic profiles and bone turnover].

The possible reasons for the inconclusive results of previous RCTs have been discussed elsewhere in detail (22,23). Here we will highlight important aspects of designing a future RCT which may provide answers to the disputed question of the efficacy of LT3+LT4 in this situation –

(1) **Selection of trial subjects** –

(a) *Presence of symptoms and threshold for recruitment* –

The primary outcome in any RCT of LT3+LT4 in hypothyroidism should be their effect on symptoms and QoL as recommended by expert bodies (22, 23). Identifiable and measurable symptoms "quantifying" an unsatisfactory response to LT4 alone should be present at recruitment despite TSH within the reference range (22,24).

This is achieved by the following –

(i) The use of thyroid specific PROMs - Most studies hitherto, have used generic, non-thyroid specific PROMs such as SF-36, HADS, BDI, and GHQ. They are not adequately responsive to changes in symptoms and QoL in benign thyroid disease (25). There is now a consensus that thyroid specific PROMs, ThyPRO and ThyPRO39 (a shorter version) are the best PROMs for use in RCTs (21,22,25). An even shorter version, the Composite Scale (utilising 22 of 39 ThyPRO39 items) shows comparable validity and reliability (26). These thyroid specific PROMs fulfil two important criteria i.e., content validity (relevant and acceptable to patients and physicians), and responsiveness (detecting subtle changes over time) (21,27-29).
However, patient preference should also be measured as PROMs alone may not accurately capture subtle differences between treatments (21), as it may be affected by the presence/absence of symptoms at recruitment, and thyroid "activity" during the RCT (e.g., LT3 causing iatrogenic hyperthyroidism). One metanalysis of crossover trials indicated that 48% preferred combination therapy (30), although another suggested there was no consistent evidence of improved preference except through chance (31).

(ii) Symptom threshold for recruitment - a ThyPRO Composite scale score of 32 or a TSQ >4 has been shown to represent a score which signifies a worse QoL which is sufficiently above the mean score in patients with hypothyroidism (32). A potential concern about applying a threshold QoL score in recruitment is that individuals with lower scores may have "normalised" their view of their QoL after many years of LT4 treatment, yet still benefit substantially from a change in therapy.

(iii) Quantifying "minimal important change" after treatment – it is important to define the clinical relevance of a change in PROM score in reference to patient perception of benefit. The term "minimal important change" (MIC) is used to define the "smallest change in score in the construct to be measured that patients perceive as important" (33-35). A change of 9 points in the ThyPRO Composite score has been identified as a valid MIC (36).

(b) *LT4 dose at recruitment* –

Hypothyroid subjects who take a minimum of 1.2μg/kg/day (21,22) or 100μg/day (for pragmatic reasons), have minimal or no endogenous thyroid function and/or derive the majority of their thyroid hormone from exogenous LT4 therapy. These individuals also have the greatest distrurbance of their circulating T3/T4 ratio (14). They may be more likely to derive benefit from thyroid hormone replacement, including LT3+LT4, than those who have residual function. This also excludes many subjects with subclinical hypothyroidism.

(c) *Cause of hypothyroidism* –

The only patients without any endogenous thyroid function are those who have had a total thyroidectomy e.g., patients with treated thyroid cancer (although such patients on suppressive LT4 therpay should be excluded). However, enrolling only them would reduce the generalisability of the results of a RCT considerably as the majority of subjects on LT4 have Hashimoto's thyroiditis. Unrestricted enrolment as regards aetiology of hypothyroidism and later subgroup analysis is a pragmatic solution.

(d) *The Thr92Ala polymorphism* –

Approximately 12-14% of the UK population are homozygous for a single nucleotide polymorphism (SNP) in the D2 gene (Thr92Ala in its ubiquitination site) (37). The exact mechanism by which Thr92Ala SNP affects D2 function is unknown as it affects residues distant to the catalytic site making diminished catalytic activity unlikely. But it may cause intracellular organelle dysfunction by abnormal translocation to the Golgi apparatus and disruption of mitochondrial function, apoptosis, and growth factor signalling (18,38). This polymorphism however has been shown to be beneficial in the general population (heterozygosity reduces acute lung injury in sepsis by 35% and reduces mortality from COVID-19 by 50%) (39,40).

The D2 SNP affects symptom load, thyroid hormone levels and response to LT3+LT4 in both animal and human studies. It reduces D2 "activity" in mouse pituitary and HEK-293 cells (38,41). Mice homozygous for the SNP showed reduced T3 activity in some brain regions associated with behavioural abnormalities (sleep more, reduced physical activity) (38). Methimazole made these abnormalities worse and they responded well to LT3+LT4.

As expected, human studies have produced significant but occasionally conflicting results – (i) a UK study showed impaired psychological well-being (GHQ) on LT4 alone in homozygotes and a greater response to LT3+LT4 compared to those with the wild type allele (42). (ii) A more recent study showed a preference for LT3+LT4 in those with either or both monocarboxylase transporter 10 and Thr92Ala-D2 polymorphisms (43). (iii) A study of thyroidectomised patients with at least one Thr92Ala allele demonstrated lower serum T3 levels with normal TSH concentrations (41). (iv) We observed that in individuals on LT4 alone (n=573), homozygosity for the Thr92Ala polymorphism was associated with reduced QoL (5). (v) A subsequent study failed to replicate our findings, but was potentially underpowered - had fewer subjects on LT4 (n=364) (44). (vi) We have replicated

our findings in the HUNT study in Norway (n=46,712, n=1100 on LT4) (45). The Thr92Ala SNP was present in 13% of the population and was not associated with increased HADS scores in subjects not taking LT4. HADS was 0.71 points higher (0.39-1.02, p<0.001) in subjects on LT4 overall, and 1.83 points higher (0.93-2.73 p<0.001) in those on LT4 who were homozygous for Thr92Ala compared to individuals not on LT4. Thr92Ala non-homozygous individuals on LT4 were 22% more likely than those not on LT4 to reach the threshold for HADS anxiety caseness, whilst homozygous individuals were 208% more likely.

We believe initial proof of efficacy should be sought in subjects homozygous for the D2 Thr92Ala SNP as this group have a 3-fold increased symptom burden. Recruiting subjects with symptoms who have the potential to respond well to combination therapy, will reduce the size (and cost) of the study and increase its power. Note, however, that even those non-homozygous for Thr92Ala have a 22% increase in symptoms and may derive benefit from LT3.

In summary, we justify selection of subjects with the D2 SNP as follows – (1) subjects homozygous for Thr92Ala have been shown in 2 independent populations to have a greater symptom burden on LT4 than non-homozygous individuals. (2) They have been shown to have a greater response to LT4+LT3 compared to non-homozygous individuals. (3) However, we have confirmed that non-homozygous individuals also have an excess of psychological morbidity on LT4 compared to the background population, confirming that all subjects with the SNP on LT4 have the potential to benefit from LT3. (4) Power calculations indicate that studies involving Thr92Ala homozygous subjects subjects allow for a 3-fold smaller study, saving on time and money.

(2) **Selecting a physiological LT3 preparation**

In subjects with preserved endogenous thyroid function, serum T3 fluctuations are narrow (46). LT3 preparations which deliver profiles which mimic normal human T3 profiles make it easier to dose adjust and may enhance safety. Current short acting LT3 preparations are unphysiological, need to be given at least two/three times per day to achieve a semblance of a physiological profile.

(a) *Current short acting LT3 preparations* –

(i) *Produce unphysiological "peaks"* - One reason why previous RCTs may have been inconclusive was the use of "unphysiological" LT3 preparations (21). Current LT3 preparations including natural desiccated thyroid (NDT) are all short acting (unpublished data)(figures 1,2). They last a few hours in the serum and produce "peaks" with persistent T3 levels above the upper limit of the reference range (ULRR) for a significant length of time – the number of peaks depending on the frequency of administration. Of the 16 RCTs for which there is information, 9 used a once/day, 4 used a twice/day and one used a three times/day LT3 regime (Table 1).

(ii) *Difficult to monitor for "dose adjustment"* – The unphysiological pharmacokinetic profiles and "peaks" so produced (Figures 1,2), do not lend themselves to monitoring of thyroid hormones for dose adjustment. Figure 1 illustrates the

variability of serum free T3 at different times pre and post oral short acting LT3, making recommendations about the timing of blood testing for dose adjustment all but impossible. The current recommendation for measuring trough free T3 levels does not consider the previously mentioned peaks, and time spent out of the reference range (ULRR) which may adversely affect patients both in the short and long term. As we recently showed in hypothyroid subjects given NDT (ERFA and Armour thyroid) there was a significant difference in time spent outside the ULRR despite similar trough free T3 concentrations (unpublished data) (figure 2).

(b) Long acting "physiological" LT3 preparations –

There have been calls from Specialist Societies to investigate long acting LT3 in RCTs in symptomatic hypothyroid subjects on LT4 (21,47). There is a shortage of long-acting preparations with clinical utility (Table 2, known preparations), but 2 preparations have undergone preliminary trials.

(i) Poly-zinc-liothyronine (PZL) –
PZL is a polymeric compound of zinc and LT3, which following oral administration adheres to the intestinal mucosa (48,49). This slows down its gut transit time and in essence forms a "depot" for slow release of T3 which is then absorbed into the blood stream (50). A phase 1 trial comparing PZL and LT3 showed a 6-hour plateau in serum T3 C_{max} for PZL which was 30% lower than for LT3. Furthermore, T3 remained over half C_{max} for more than 24 hours (51). This preparation may produce more physiological T3 pharmacokinetics. It has not currently been tested in QoL studies.

(iii) Triiodothyronine sulphate (T3S) - Sulphation of the hydroxyl group of the T3 molecule generates T3 sulphate (T3S) which is an inactive molecule, naturally formed during human thyroid hormone metabolism. This targets T3 for destruction by deiodinase 1 (D1). But, if required (e.g., in hypothyroidism), T3S can be desulphated by local sulphatase enzymes (52,53) or the gut microbiome (54) to regenerate "active" T3.

Sulphation also confers the following qualities on T3S –
(1) increased solubility targets it for biliary excretion and reabsorption in an enterohepatic circulation. Sulphation and desulphation enzymes are highly expressed in the liver.
(2) T3S is markedly preferred as a substrate over T3 by the D1 deiodinase enzyme expressed in the liver, kidney and intestine (55).
(3) D1 activity is increased in hyperthyroidism and D2 activity is reduced.

Taken together, these elements create a self-regulating system to stabilise serum T3 levels - if T3 rises, more is sulphated and excreted via the liver and kidney and increased D1 levels at these sites result in irreversible destruction of T3S to inactive metabolites. By contrast, in hypothyroidism when T3 levels are low, D1 activity is low in the liver and intestine and more of the T3S excreted in the bile can be reabsorbed and desulphated to restore T3 levels.

When T3S is administered orally, this system generates a favorable pharmacokinetic profile: T3S levels peak and return to baseline in 12 hours, but

then (desulphated) serum T3 levels rise and remain high for over 48 hours, likely through enterohepatic recirculation and desulphation. The result is in effect a "slow-release preparation", as demonstrated in thyroidectomized humans in 2014 (56). Santini's group also showed in a continuous dosing study over 11 weeks that replacing 25ug of T4 with 40ug of T3S in subjects on LT4 monotherapy, results in a reduction in serum free T4 without a reduction in free T3. This restores a physiological T4/T3 ratio alongside a normal TSH in 89% (n=36) as compared to fewer than 45% on LT4 alone (57). A particularly attractive feature of T3S therapy is that by exploiting natural physiology it is self-regulating (as described above), with excess levels being rapidly destroyed and/or excreted, generating a very wide therapeutic index and level of safety. Consistent with this, no safety signals were observed in the 11-week study, which was uncontrolled and not designed or powered for clinical endpoints (57).

(3) **Appropriate Trial Design**

RCTs provide high quality evidence with minimal bias. But they are difficult to design and execute, expensive, time consuming and targeted at specific groups (may therefore be difficult to extrapolate to other groups or to generalise their results).

However, several broad principles apply. Such a trial should be randomised (thereby matched for sex, menopausal status, age etc.), blinded to both participant and investigator (e.g., participant's thyroid hormone status) and placebo controlled (over-encapsulation or identical preparations). All laboratory assessments should be done in a single reference laboratory to minimise inter- and intra-assay variations and preferably in a single batch (although this may not be practical). However, safety tests and thyroid tests for dose adjustment may be done locally.

A long study duration of 12 – 24 months is recommended in view of the long half-life of LT4 (approximately 7 days), time taken to achieve a "steady state" for dose adjustment, and to assess medium term efficacy and safety e.g., on cardiac and bone tissue. However, a long trial may affect enrolment adversely and increase dropout rates as well as increasing cost.

Of the various trial designs available, a crossover design is attractive to participants as they are certain to receive the intervention/drug under investigation during one of the trial phases. Furthermore, the ability to do paired analyses enhances its statistical power reducing the number needed to be enrolled and reducing costs. However, "carryover" of effects of one therapy to a different phase of the study, loss and inability to analyse data if patients drop out are disadvantages. These effects maybe mitigated if a parallel group design is adopted.

Conclusions

The issue of "optimal" treatment for hypothyroidism remains unresolved. LT4 monotherapy satisfies the majority, but a significant minority remain dissatisfied because of persistent symptoms impairing their QoL. A trial of LT3 is recommended in them by international specialist societies (58-60).

Although the theoretical evidence for this recommendation is convincing, evidence of proof of efficacy from RCTs is less so. However, some limitations of these RCTs should be addressed in future trials - they (i) were underpowered; (ii) used inappropriate outcome measures; (iii) were mainly of short duration; and (iv) used short acting, unphysiological LT3 preparations. We believe the availability of long acting LT3 preparations such as PZL and T3S, overcomes this disadvantage and their efficacy should be investigated in phase 2b/3 RCTs.

We recommend that in such trials, researchers study subjects with a high symptom burden with a propensity to responding better to combination LT3+LT4 therapy (Box 1). In this regard subjects with the D2 polymorphism (Thr92Ala) would be ideal as they fulfil both criteria. There is a plausible biological role for the D2 polymorphism in determining symptom load and also a better response to LT3+LT4 treatment. This strategy eliminates subjects with the potential for only a minimal, clinically insignificant response to LT3 (e.g. those with subclinical hypothyroidism).

A placebo controlled, double blind, cross over RCT would be ideal because of the advantages of such a design – adequately powered with limited numbers, all subjects receiving the active and the placebo medications and limiting costs within acceptable limits.

References –

1. Millan-Alanis JM, Gonza´lez JG, Flores-Rodr´ıguez A, Singh Ospina N, Maraka S, Moreno-Pena PJ et al. RR. Benefits and Harms of Levothyroxine/L-Triiodothyronine Versus Levothyroxine Monotherapy for Adult Patients with Hypothyroidism: Systematic Review and Meta-Analysis. Thyroid 2021; 11: 1613-25. DOI: 10.1089/thy.2021.0270
2. Hoang TD, Olsen CH, Mai VQ, Clyde PW, Shakir MKM. Desiccated thyroid extract compared with levothyroxine in the treatment of hypothyroidism: a randomized, double-blind, crossover study. J Clin Endocrinol Metab 2013; 98:1982–1990.
3. Slawik M, Klawitter B, Meiser E, Schories M, Zwermann O, Borm K, et al. Thyroid hormone replacement for central hypothyroidism: a randomized controlled trial comparing two doses of thyroxine (T4) with a combination of T4 and triiodothyronine. J Clin Endocrinol Metab 2007; 92: 4115–4122.
4. Wekking EM, Appelhof BC, Fliers E, Schene AH, Huyser J, Tijssen JG, et al. Cognitive functioning and wellbeing in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. *Eur J Endocrinol*. 2005;153:747-753.
5. Saravanan P, Chau WF, Roberts N, Vedhara K, Greenwood R, Dayan CM. Psychological well-being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. *Clin Endocrinol (Oxf)*. 2002;57:577-585.
6. Panicker V, Evans J, Bjoro T, Asvold BO, Dayan CM, Bjerkeset O. A paradoxical difference in relationship between anxiety, depression and thyroid function in subjects on and not on T4: findings from the HUNT study. *Clin Endocrinol (Oxf)*. 2009;71:574-580.
7. Taylor PN, Iqbal A, Minassian C, Sayers A, Draman MS, Greenwood R, et al. Falling threshold for treatment of bor- derline elevated thyrotropin levels-balancing benefits and risks: evidence from a large community-based study. JAMA Intern Med 2014; 174:32-9.
8. Medici BB, Nygaard B, la Cour JL, Grand MK, Siersma V, Nicolaisdottir DR, et al. Changes in prescription routines for treating hypothyroidism between 2001 and 2015: an observational study of 929,684 primary care patients in Copenhagen. Thyroid 2019; 29: 910-9.
9. Wyne KL, Nair L, Schneiderman CP, Pinsky B, Antunez Flores O, Guo D et al. Hypothyroidism Prevalence in the United States: A Retrospective Study Combining National Health and Nutrition Examination Survey and Claims Data, 2009–2019. J Endocr Soc, 2023, 7, 1–11. https://doi.org/10.1210/jendso/bvac172
10. Younes YR, Perros P, Hegedüs L, Papini E, Nagy EV, Attanasio R et al. Use of thyroid hormones in hypothyroid and euthyroid patients: A THESIS questionnaire survey of UK endocrinologists. Clin Endocrinol (Oxf). 2023; 98: 238-248. doi: 10.1111/cen.14812.
11. Yu Ning[1], Yun J. Cheng[2], Li J. Liu[2], Jaskanwal D. S. Sara[3], Zhi Y. Cao[4], Wei P. Zheng et al. What is the association of hypothyroidism with risks of cardiovascular events and mortality? A meta-analysis of 55 cohort studies involving 1,898,314 participants. BMC Medicine 2017; 15:21 DOI 10.1186/s12916-017-0777-9
12. Samuels MH, Kolobova I, Smeraglio A, Peters D, Purnell JQ, Schuff KG. Effects of levothyroxine replacement or suppressive therapy on energy expenditure and body composition. Thyroid 2016; 26: 347–355.

13. McAninch EA, Rajan KB, Miller CH, Bianco AC. Systemic thyroid hormone status during levothyroxine therapy in hypothyroidism: a systematic review and meta-analysis. J Clin Endocrinol Metab 2018; 103:4533–4542.
14. Gullo D, Latina A, Frasca F, Le Moli R, Pellegriti G et al. Levothyroxine Monotherapy Cannot Guarantee Euthyroidism in All Athyreotic Patients. PLoS ONE 2011; 6: e22552. doi:10.1371/journal.pone.0022552;
15. Ettleson MD, Bianco AC. Individualized Therapy for Hypothyroidism: Is T4 Enough for Everyone? J Clin Endocrinol Metab 2020, 105: e3090–e3104. https://academic.oup.com/jcem doi:10.1210/clinem/dgaa430
16. Perros P, Van Der Feltz-Cornelis C, Papini E, Nagy EV, Weetman AP, Hegedus L. The enigma of persistent symptoms in hypothyroid patients treated with levothyroxine: A narrative review. Clin Endocrinol 2023; 98: 461-68
17. Escobar-Morreale HF, Obregon MJ, Escobar del Rey F, Morreale de Escobar G. Replacement therapy for hypothyroidism with thy- roxine alone does not ensure euthyroidism in all tissues, as stud- ied in thyroidectomized rats. *J Clin Invest*. 1995;96:2828-2838
18. Werneck de Castro JP, Fonseca TL, Ueta CB, McAninch EA, Abdalla S, Wittmann G et al. Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. *J Clin Invest*. 2015;125:769-781.
19. Christoffolete MA, Ribeiro R, Singru P, Fekete C, da Silva WS, Gordon DF et al., Atypical expression of type 2 iodothyronine deiodinase in thyrotrophs explains the thyroxine-mediated pituitary thyrotropin feedback mechanism. Endocrinology. 2006 Apr;147:1735-43. doi: 10.1210/en.2005-1300. Epub 2006 Jan 5. PMID: 16396983.
20. Watt T. Measuring impact of benign thyroid diseases on quality of life. In: Caplan M, editor. Reference Module in Biomedical Scienc- es. Amsterdam: Elsevier; 2018.
21. Wong CK, Lang BH, Lam CL. A systematic review of quality of thyroid-specific health- related quality-of-life instruments recommends ThyPRO for patients with benign thyroid diseases. J Clin Epidemiol. 2016 Oct;78: 63–72.
22. Jonklaas J, Bianco AC, Cappola AR, Celi FS, Fliers E, Heuer H et al. Evidence-Based Use of Levothyroxine/Liothyronine Combinations in Treating Hypothyroidism: A Consensus Document. Thyroid 2021; 31: 156-182;
23. Cappola AR. Design of the Optimal Trial of Combination Therapy. Front Endocrinol (Lausanne). 2020 Apr 3;11:168. doi: 10.3389/fendo.2020.00168. PMID: 32318021; PMCID: PMC7146056.
24. Vasileiou M, Gilbert J, Fishburn S, Boelaert K, Guideline Committee. Thyroid disease assessment and management: summary of NICE guidance. Br Med J. 2020 Jan 29;368:m41. doi: 10.1136/bmj.m41.
25. Watt T, Cramon PK, Hegedüs L, Bjorner JB, Bonnema SJ, Rasmussen ÅK et al., The thyroid- related quality of life measure ThyPRO has good responsiveness and ability to detect relevant treatment effects. *J Clin Endocrinol Metab* 2014; 99**:** 3708–3717. (https://doi. org/10.1210/jc.2014-1322)
26. Watt T, Bjorner JB, Groenvold M, Cramon P, Winther KH, Hegedüs L, et al. Development of a Short Version of the Thyroid-Related Pa- tient-Reported Outcome ThyPRO. Thyroid. 2015; 25:1069–79.
27. Watt T, Hegedüs L, Groenvold M, Bjorner JB, Rasmussen AK, Bonnema SJ & Feldt-Rasmussen U. Validity and reliability of the novel thyroid-specific quality of life questionnaire, ThyPRO. *Eur J Endocrinol* 2010; 162**:** 161–167. (https://doi.org/10.1530/ EJE-09-0521)

28. Watt T, Groenvold M, Hegedüs L, Bonnema SJ, Rasmussen ÅK, Feldt-Rasmussen U et al. Few items in the thyroid-related quality of life instrument ThyPRO exhibited differential item functioning. *Qual Life Res* 2014; 23**:** 327–338. (https://doi.org/10.1007/ s11136-013-0462-1)
29. Watt T, Groenvold M, Deng N, Gandek B, Feldt-Rasmussen U, Rasmussen ÅK et al. Confirmatory factor analysis of the thyroid-related quality of life questionnaire ThyPRO. *Health and Quality of Life Outcomes* 2014; 12**:** 126. (https:// doi.org/10.1186/s12955-014-0126-z)
30. Wiersinga WM, Duntas L, Fadeyev V, Nygaard B, Vanderpump MP. 2012 ETA Guidelines: The Use of L-T4 + L-T3 in the Treatment of Hypothyroidism. Eur Thyroid J. 2012; 1: 55–71. https://www.nice.org.uk/guidance/ng145/evidence;
31. Akirov A, Fazelzad R, Ezzat S, Thabane L, Sawka AM. A Systematic Review and Meta-Analysis of Patient Preferences for Combination Thyroid Hormone Treatment for Hypothyroidism. Front Endocrinol (Lausanne). 2019; 24;10:477. doi: 10.3389/fendo.2019.00477. PMID: 31396154; PMCID: PMC6667836.
32. Winther KH, Cramon P, Watt T, Bjorner JB, Ekholm O, Feldt-Rasmussen U, et al. Disease-Specific as Well as Generic Quality of Life Is Widely Impacted in Autoimmune Hypothyroidism and Improves during the First Six Months of Levothyroxine Therapy. PLoS One. 2016;11:e0156925. doi: 10.1371/journal.pone.0156925. eCollection 2016.
33. Prinsen CAC, Mokkink LB, Bouter LM, Alonso J, Patrick DL, de Vet HCW et al. COSMIN guideline for systematic reviews of patient reported outcome measures. *Qual Life Res* 2018; 27**:** 1147–1157. (https://doi.org/10.1007/s11136-018-1798-3)
34. Vet HCW De, Terwee CB, Mokkink LB & Knol DL. *Measurement in Medicine*. Cambridge University Press, New York. 2011.
35. Mokkink LB, Terwee CB, Knoll DL, Stratford PW, Alonso J, Patrick DL et al. The COSMIN checklist for evaluating the methodological quality of studies on measurement properties: a clarification of its content. *BMC Medical Research Methodology* 2010; 10**:** 22 (available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L 360294071). (https://doi.org/10.1186/1471-2288
36. Nordqvist SF, Boesen VB, Rasmussen AK, Feldt-Rasmussen U, Hegedüs L:, Bonnema SJ et al. Determining minimal important change for the thyroid related quality of life questionnaire ThyPRO. Endo Connect 2021; 10: 316–324
37. Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM et al., Common variation in the DIO2 gene predicts baseline psychological wellbeing and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. J Clin Endocrinol Metab. 2009;94:1623–9.
38. Jo S, Fonseca TL, Bocco BM, Fernandes GW, McAninch EA, Bolin AP, et al. Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. J Clin Invest. 2019; 129:230–45
39. Ma SF, Xie L, Pino-Yanes M, Sammani S, Wade MS, Letsiou E, et al. Type 2 deiodinase and host responses of sepsis and acute lung injury. *Am J Respir Cell Mol Biol.* 2011;45:1203-1211.
40. de Lima Beltrão FE, de Almeida Beltrão DC, Carvalhal G, de Lima Beltrão FE, de Souza Braga Filho J, de Brito Oliveira J et al. Heterozygote Advantage of the Type II Deiodinase Thr92Ala Polymorphism on Intrahospital Mortality of COVID-19. J Clin Endocrinol Metab. 2022; 17:107:e2488-e2501. doi: 10.1210/clinem/dgac075.

41. Castagna MG, Dentice M, Cantara S, Ambrosio R, Maino F, Porcelli T et al. DIO2 Thr92Ala Reduces Deiodinase-2 Activity and Serum T3 Levels in Thyroid Deficient Patients. J Clin Endocrinol Metab. 2017;102: 1623–30
42. Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, et al. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. J Clin Endocrinol Metab. 2009;94:1623–9.
43. Nygaard B, Jensen EW, Kvetny J, Jarløv A, Faber J. Effect of combination therapy with thyroxine (T4) and 3,5,3′-triiodothyronine versus T4 monotherapy in patients with hypothyroidism, a double-blind, randomised cross-over study. Eur J Endocrinol. 2009; 161:895–902.
44. Wouters HJ, van Loon HC, van der Klauw MM, Elderson MF, Slagter SN, Kobold AM et al. No Effect of the Thr92Ala Polymorphism of Deiodinase-2 on Thyroid Hormone Parameters, Health-Relat- ed Quality of Life, and Cognitive Functioning in a Large Population-Based Cohort Study. Thyroid. 2017;27:147–155.
45. Taylor P, Haug E, Heald A, Premawardhana L, Okosieme O, Stedman M et al., Individuals on levothyroxine have higher HADS anxiety and depression scores than the general population and this is exacerbated by the Thr92Ala substitution in DIO2. Endocrine Abstracts (2022) 86 EC1.3 | DOI: 10.1530/endoabs.86.EC1.3
46. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T4 and T3 in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab. 2002;87: 1068–72
47. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid associ- ation task force on thyroid hormone replacement. Thyroid 2014; 24:1670–1751.
48. Price JD, Piccariello T, Palmer S Metal-coordinated pharmaceuticals. Drug Dev Deliv 2014; 14:34–39.
49. Smart JD. The basics and underlying mechanisms of mucoadhesion. Adv Drug Deliv Rev 2005; 57:1556–1568.
50. Da Conceicao RR, Fernandes GW, Fonseca TL, Bocco B, Bianco AC. Metal coordinated poly-zinc-liothyronine provides stable circulating triiodothyronine levels in hypothyroid rats. Thyroid 2018; 28:1425–1433
51. Dumitrescu, AM, Hanlon EC, Arosemena M, Duchon O, Ettleson M, Giurcanu M et al,. AC. Extended Absorption of Liothyronine from Poly-Zinc-Liothyronine: Results from a Phase 1, Double-Blind, Randomized, and Controlled Study in Humans. Thyroid. 2022; 32: 96-205. doi: 10.1089/thy.2021.0304. .
52. Kung MP, Spaulding SW, Roth JA. Desulfation of 3,5,3- triiodothyronine sulfate by microsomes from human and rat tissues. Endocrinol 1988;122:1195–1200; doi: 10.1210/endo-122-4-1195
53. Santini F, Chopra IJ, Wu SY. Solomon DH, Chua Teco GN. Metabolism of 3,5,3-triiodothyronine sulfate by tissues of the fetal rat: A consideration of the role of desulfation of 3,5,3- triiodothyronine sulfate as a source of T3. Pediatr Res 1992;31:541–544; doi: 10.1203/00006450-199206000- 00001
54. Hazenberg MP, de Herder WW, Visser TJ. Hydrolysis of iodothyronine conjugates by intestinal bacteria. FEMS Microbiol Rev 1988;4:9–16; doi: 10.1111/j.1574-6968.1988.tb02709.x-i1
55. Paul A. Foster. Steroid Sulphatase and Its Inhibitors: Past, Present, and Future. Molecules 2021, 26, 2852. https://doi.org/10.3390/molecules26102852

56. Santini. T3 Concentrations for 48 Hours Following T3S. Endocrine Practice 2014, 20, 680– 689.
57. Santini. Treating Hypothyroid Patients With L-T4+T3S. Frontiers Endocrinol, 2019 10, 826.
58. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association Task Force on thyroid hormone replacement. Thyroid. 2014;4: 1670-1751. doi:10.1089/thy.2014.0028
59. Wiersinga WM, Duntas L, Fadeyev V, Nygaard B, Vanderpump MPJ. 2012 ETA guidelines: the use of L-T4 + L-T3 in the treatment of hypothyroidism. Eur Thyroid J. 2012;1:55-71. doi:10.1159/ 000339444
60. Ahluwalia R, Baldeweg SE, Boelaert K, et al. Use of liothyronine (T3) in hypothyroidism: Joint British Thyroid Association/Society for endocrinology consensus statement. Clin Endocrinol (Oxf). 2023; 99: 206-216. doi:10.1111/cen.14935
61. Saravanan P, Siddique H, Simmons DJ, Greenwood R, Dayan CM. Twenty-four hour hormone profiles of TSH, Free T3 and free T4 in hypothyroid patients on combined T3/T4 therapy. Exp Clin Endocrinol Diabetes. 2007;115:261–7.
62. Alomari M, Vuddanda PR, Trenfield SJ, DoDoo CC, Velega S, Basit AW et.al Printing T3 and T4 oral drug combinations as a novel strategy for hypothyroidism. Int J Phar 2018; 549: 363-9
63. Bakhteyar H, Cassone C, Kohan HG, Sani SN. Kinetic analysis of drug release from compounded slow-release capsules of liothyronine sodium (T3). *Int J Pharm Compd.* (2017) 21:418–25.
64. Hennemann G, Docter R, Visser TJ, Postema PT, Krenning EP. Thyroxine plus low-dose, slow-release triiodothyronine replacement in hypothyroidism: proof of principle. *Thyroid Offic J Am Thyroid Assoc.* (2004) 14:271–5. doi: 10.1089/105072504323030924
65. Jonklaas J, Burman KD, Wang H, Latham KR. Single-dose T3 administration: kinetics and effects on biochemical and physiological parameters. *Ther Drug Monit.* (2015) 37:110–8. doi: 10.1097/FTD.00000000000 00113
66. Dumitrescu AM, Hanlon EC, Arosemena M, Duchon O, Ettleson M, Giurcanu M, Bianco AC. Extended Absorption of Liothyronine from Poly-Zinc-Liothyronine: Results from a Phase 1, Double-Blind, Randomized, and Controlled Study in Humans. *Thyroid*. 2022 Feb;32(2):196-205. doi: 10.1089/thy.2021.0304. Epub 2021 Dec 31
67. Bianco AC, Anderson G, Forrest D, Galton VA, Gereben B, Kim BW, et al. American thyroid association guide to investigating thyroid hormone economy and action in rodent and cell models. *Thyroid*. (2014) 24:88–168. doi: 10.1089/thy.2013.0109
68. Sreedharan S, Patel R. Long-term sustained delivery of liothyronine (L- T3) with subdermal proneuratm implants. In: *87th Annual Meeting of the American Thyroid Association* Vancouver, BC (2017).
69. Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, et al. Generation of functional thyroid from embryonic stem cells. *Nature.* (2012) 491:66–71. doi: 10.1038/nature11525
70. Kurmann AA, Serra M, Hawkins F, Rankin SA, Mori M, Astapova I, et al. Regeneration of thyroid function by transplantation of differentiated pluripotent stem cells. *Cell Stem Cell.* (2015) 17:527–42. doi: 10.1016/j.stem.2015. 09.004

71. Finan B, Clemmensen C, Zhu Z, Stemmer K, Gauthier K, Muller L, et al. Chemical hybridization of glucagon and thyroid hormone optimizes therapeutic impact for metabolic disease. *Cell.* (2016) 167:843–57 e14. doi: 10.1016/j.cell.2016.09.014
72. Mdzinarishvili A, Sutariya V, Talasila PK, Geldenhuys WJ, Sadana P. Engineering triiodothyronine (T3) nanoparticle for use in ischemic brain stroke. *Drug Deliv Transl Res.* (2013) 3:309–17. doi: 10.1007/s13346-012- 0117-8

Tables

Author (year)	**Country**	**Design**	**Duration**	**N (% women)**	**Dose**	**Diagnosis**
1.Bunevicius (2000)	Lithuania	Crossover	5 W	26 (100)	o.d.	AITD+ThyCa *
2. Bunevicius (2002)	Lithuania	Crossover	5 W	13 (100)	o.d.	Subtotal Thy
3. Clyde (2003)	USA	Parallel	4 M	46 (82)	b.d.	AITD+post ablative
4. Sawka (2003)	USA	Parallel	15 W	40 (90)	b.d.	AITD
5. Walsh (2003)	Australia	Crossover	10 W	110 (93)	o.d.	AITD+post ablative
6. Siegmund (2004)	Germany	Crossover	12 W	26	ND	AITD
7. Appelhof (2005)	Croatia	Parallel	15 W	141 (85)	b.d.	AITD
8. Esco-Morreale (2005)	Spain	Crossover	8 W	28 (100)	o.d.	AITD+post ablative
9. Rodriguez (2005)	USA	Crossover	6 W	30 (89)	o.d.	AITD+post ablative
10. Fadeyev (2005)	Russia	Parallel	6 M	58 (100)	o.d.	AITD
11. Saravanan (2005)	UK	Parallel	12 M	584 (84)	o.d.	ND
*12. Slawik (2007)**	*Germany*	*Crossover*	*5 W*	*32 (8)*	*o.d.*	*Central hypo*
13. Nygaard (2009)	Denmark	Crossover	12 W	68 (93)	o.d.	AITD
14. Valizadeh (2009)	Iran	Parallel	16 W	71 (80)	b.d.	AITD+post ablative
15. Fadeyev (2010)	Russia	Parallel	24 W	36 (100)	o.d.	AITD
*16. Hoang (2013)****	*USA*	*Crossover*	*22 W*	*78 (75)*	*o.d.*	*NDT*
17. Kaminski (2016)	Brazil	Crossover	8 W	32 (94)	o.d.	AITD
18. Krysiak (2018)	Poland	Quasi Rand	24 W	39 (100)	ND	Partial Thyroidectomy

Table 1 – Clinical Trials of LT3+LT4 combination therapy

Preparation	Details of action if known	Current status
Oral/Gastrointestinal		
T3 granules		No information
Orodispersible films (62)	Thermal inject printing deposits LT4 and LT3 to water soluble films	Pharmacokinetics not done
T3 and swellable hydrophilic matrix (63)	T3 combined with hydrophilic swellable matrix – rate of release adjusted according to grade of Methocel and SimpleCap/Lactose matrix in capsules	First study showed lower peak T_{max} prolonged from 3.2 to 5 hours (64) Second study using cellulose and Mg stearate matrix was unsuccessful (65) Current status unknown
PZL (L-thyroxine polymer with zinc)	T3 polymer with Zn – PZL. Increased gut mucoadhesion forming drug "depot" for slow release	Successful animal and Phase 1 studies (66) Awaiting regulatory approvals
T3 sulfate (T3S)	T3S desulphated by tissue and gut microbiome sulphatases providing a favourable longacting pharmacokinetic profile	Two phase 1 human studies completed (56, 57). Phase 2b RCT funded (MR/X013170/1) and planned
Parenteral		
Aqueous and oily T3 preparations		Inconsistent results in animal studies. Current status unknown
Osmotic subcutaneous mini pumps/pellets with T3 (67)	Release T3 over many days	Animal studies successful. Current status unknown
Implantable subcutaneous min-rods with T3 (68)	Stable long term T3 release	Sucessful animal studies. Current status unknown
Intraperitoneal and intravenous T3 preparations (67)		Produce unphysiological T3 peaks. Current status unknown
Regenerative Methods		
Mice Stem cells expressing NKX.2 and PAX 8 grafted onto mice (69,70)	Generates thyroid cells from stem cells leading to thyroid follicular cells when implanted in to mice	No human studies done. Current status unknown
Tissue Targeting		
T3 conjugated with glucagon (71) and the use nanotechnology to deliver T3 to the brain (72)	Conjugates of glucagon and T3 delivers to the liver	No human studies. Current status unknown

Table 2 – Known long-acting T3 preparations and their current availabiity

Key elements of a trial of combined LT3+LT4 therapy in hypothyroidism

Aim – To provide phase 2b/3 efficacy data that combined LT3+LT4 therapy is superior to LT4 alone

Primary outcome –

Symptom improvement using a thyroid specific PROM e.g. ThyPRO39 or its composite score

Secondary outcomes –

1. pharmacokinetic profiles of interventional drug
2. changes to weight/BMI, body composition, REE, metabolomic profiles and bone turnover].
3. safety outcomes

Patient selection –

1. presence of symptoms and threshold for recruitment
2. use of thyroid specific PROMs
3. recognising minimal important change in score
4. higher symptom load and greater response to LT3 e.g. deiodinase 2 snp

LT4 dose at recruitment –

Minimum of 1.2μg/kg/day or 100μg/day (for pragmatic reasons)

Cause of hypothyroidism –

Unrestricted recruitment of subjects with high symptom load and a propensity to respond to treatment

Choice of interventional medication –

Long acting LT3 preparations e.g., PZL or T3S

Appropriate Trial design –

Randomised placebo-controlled trial using a cross over design

Box 1 – Key elements of a trial of combined LT3+LT4 therpy in hypothyroidism

Legends

Tables

Table 1 – Trials of LT3+LT4 clinical trials

All the above trials included subjects with autoimmune thyroid disease (AITD) except for 12 (Slawik) which recruited subjects with central hypothyroidism and 16 (Hoang) which used NDT (natural dessicated thyroid) as the interventional medication. * Trial contained thyroid cancer patients.

Table 2 – Longacting LT3 preparations currently available or under investigation

The above preparations are currently available or have being investigated.

Box 1 - Key elements of a trial of combined LT3+LT4 therpy in hypothyroidism

PROM (patient reported outcome measures); snp (single nucleotide polymorphism)

Figures

Figure 1 – 24 hour Free T3 profiles in hypothyroid subjects

Mean free T3 profiles after giving either LT4 alone (controls) or LT3+LT4 (patients) in 10 subjects with hypothyroidism. [Reprinted with permission from Saravanan et al. (61)]

Figure 2 – Serum Free T3 concentrations in hyhpothyroid subjects given natural dessicated thyroid (NDT)

Both ERFA and Armour thyroid caused FT3 concentrations to be elevated above the upper limit of the reference range (ULRR) for significant amounts of time (Premawardhana L.D. et al. Oral Presentation, British Thyroid Association, May 2021)