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# Tissue Plasminogen Activator or Perfluoropropane for Submacular Hemorrhage in Age-Related Macular Degeneration
## A Factorial Randomized Clinical Trial

George S. P. Murphy, MB BCh BAO; Azahir Saleh, MBBS; Salma Ayis, PhD; Muhammad Raza Cheema, MRes; Alex Mehta, MBBS; David H. Steel, MD; Luke Membrey, MB, BS; Mark Costen, MB, BS; Timothy L. Jackson, PhD, MBChB

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Supplemental content

**IMPORTANCE** Evidence is limited to support therapies to treat submacular hemorrhage (SMH) secondary to neovascular age-related macular degeneration (AMD) as an adjunct to anti–vascular endothelial growth factor therapy (anti-VEGF).

**OBJECTIVE** To determine if intravitreal tissue plasminogen activator (TPA) or gas improves visual acuity or promotes resolution of SMH secondary to neovascular AMD in eyes treated with ranibizumab.

**DESIGN, SETTING, AND PARTICIPANTS** This was a double-masked, sham-controlled, factorial randomized clinical trial and feasibility study that recruited participants from June 2014 to March 2019, with 12 months' follow-up. Included in the trial were patients from 4 UK vitreoretinal units who had fovea-involving SMH of at least 1 disc area secondary to neovascular AMD and were evaluated within 14 days of onset.

**INTERVENTIONS** Study eyes received baseline ranibizumab and were then randomized 2:1:1:1 to 1 of 4 intravitreal treatments: sham injection, perfluoropropane ($C_3F_8$), TPA, or combined $C_3F_8$ and TPA ($C_3F_8$ + TPA). All eyes received monthly pro re nata ranibizumab therapy over 12 months. Outcome assessors were masked to intervention assignment.

**MAIN OUTCOME AND MEASURE** Best-corrected visual acuity (BCVA) at month 3.

**RESULTS** Fifty-three of 56 participants (95%; mean [SD] age, 81.5 [8.1] years; 33 female [59%]) reached the primary end point. Study eyes were randomized to the following intravitreal treatments: sham injection (n = 23), $C_3F_8$ (n = 11), TPA (n = 11), or $C_3F_8$ + TPA (n = 11). On factorial analysis, the combined TPA groups had significantly better month 3 mean logMAR BCVA than those not receiving TPA: 0.66 vs 0.98 ($\mu_d$ = −0.32; 95% CI, −0.58 to −0.07; $P$ = .02). There was no statistically significant difference comparing groups that did vs did not receive $C_3F_8$: 0.80 vs 0.90 ($\mu_d$ = −0.11; 95% CI, −0.37 to 0.16; $P$ = .43). The combined TPA groups were less likely to have SMH present at month 1 (10 of 18 [55.6%] vs 21 of 24 [87.5%]; $P$ = .03), a benefit not evident in the combined gas groups. The mean logMAR BCVA at 3 months was not significantly different between the groups: monotherapy control, 0.99; $C_3F_8$, 0.97 (vs control $\mu_d$ = −0.02; 95% CI, −0.48 to 0.44); TPA, 0.70 (vs control $\mu_d$ = −0.29; 95% CI, −0.79 to 0.21); combined $C_3F_8$ and TPA, 0.71 (vs control $\mu_d$ = −0.36; 95% CI, −0.82 to 0.11); $P$ = .11. No safety differences were identified across the treatment groups.

**CONCLUSIONS AND RELEVANCE** Results of this randomized clinical trial suggest that TPA may increase the chance of visual acuity gain when added to ranibizumab therapy for neovascular AMD in eyes with SMH, warranting consideration of additional clinical trials.

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**Author Affiliations:** King's Ophthalmology Research Unit, Ophthalmology Department, King's College Hospital, London, United Kingdom (Murphy, Saleh, Jackson); Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom (Murphy, Ayis, Jackson); Sunderland Eye Infirmary, Sunderland, UK (Cheema, Mehta, Steel); Biosciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom (Cheema, Mehta, Steel); Maidstone NHS foundation trust, Maidstone, United Kingdom (Membrey); Hull Royal Infirmary, Hull, United Kingdom (Costen).

**Corresponding Author:** Timothy L. Jackson, PhD, MBChB, King's Ophthalmology Research Unit, Ophthalmology Department, King's College Hospital, King's College London, London SE5 9RS, United Kingdom (t.jackson1@nhs.net).

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Age-related macular degeneration (AMD) remains the leading cause of sight loss in many high-income nations.[1] Neovascular AMD can rarely be associated with large submacular hemorrhage (SMH), occurring in only 5.4 cases per million per annum.[2] Untreated, SMH leads to permanent and severe loss of vision due to rapid photoreceptor injury,[3] typically ranging from 20/100 to light perception.[4]

The anti-vascular endothelial growth factor (anti-VEGF) registration trials excluded patients with large SMHs, but there are subsequent retrospective and 1 prospective case series indicating that anti-VEGF therapy offers outcomes far better than the natural history.[5-8] Intravitreal gas tamponade has been used to try and displace the SMH, often in combination with tissue plasminogen activator (TPA) to liquify the clot and facilitate displacement.[9-14] The TPA can be administered by intravitreal injection or subretinally via a pars plana vitrectomy.

An exploratory randomized clinical trial (RCT) of 24 participants compared intravitreal TPA, gas, and bevacizumab vs vitrectomy, subretinal TPA, gas, and bevacizumab. It was not powered to detect a statistically significant difference and concluded that larger studies are needed.[15]

Another trial randomized 90 participants to intravitreal TPA, gas, and aflibercept or vitrectomy, subretinal TPA, gas, and aflibercept.[16] The study failed to show superiority in the primary end point, with a mean 16 letter gain in both groups at month 3.

The current intravitreal TPA and Perfluoropropane ($C_3F_8$) for the Treatment of Neovascular AMD With Associated SMH (TAPAS) study assessed the safety and efficacy of ranibizumab combined with intravitreal TPA, $C_3F_8$ tamponade, or both TPA and $C_3F_8$ combined ($C_3F_8$ + TPA). It aimed to determine which of these elements was driving any treatment response, to establish if a definitive study was justified and feasible, and to inform its design.

## Key Points

**Question** Does intravitreal tissue plasminogen activator (TPA) or perfluoropropane improve visual acuity or promote resolution of submacular hemorrhage secondary to neovascular age-related macular degeneration (AMD) in eyes treated with ranibizumab?

**Findings** In this factorial randomized clinical trial of 56 participants, all of whom received ranibizumab, adjunctive treatment with TPA, but not perfluoropropane, resulted in significantly better visual acuity at the month 3 primary outcome.

**Meaning** These results suggest that TPA may increase the chance of visual acuity gain when added to ranibizumab therapy for neovascular AMD in eyes with submacular hemorrhage, warranting consideration of additional clinical trials.

## Methods

The TAPAS study was a multicenter, double-masked, sham-controlled, factorial RCT and feasibility trial. It received ethical review from the London-Central Research Ethics Committee (12/LO/1928) and complied with the tenets of the declaration of Helsinki. All participants provided written informed consent. No payment or financial incentive was provided to participants. The study protocol is available in Supplement 1. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines.

### Study Population

From June 25, 2014, to March 31, 2019, participants were recruited from 4 UK National Health Service hospitals. Eligible participants had SMH, with onset within 2 weeks of screening and secondary to treatment-naive or previously treated neovascular AMD or idiopathic polypoidal choroidal vasculopathy, foveal hemorrhage of at least 1 disc area, and obscuring retinal pigment epithelium detail. Eligibility criteria are listed in the eMethods in Supplement 2. Information on participant race and ethnicity was not collected in the study database.

### Randomization and Masking

Participants were randomly allocated 2:1:1:1 to 1 of 4 groups: control, $C_3F_8$, TPA, or $C_3F_8$ + TPA, respectively, using randomly permuted blocks. The statisticians developed an allocation list, with groups randomly allocated in a listwise manner. The list was maintained in the sponsor's clinical trials pharmacy, and an unmasked pharmacist allocated treatment according to the block order. The treating investigator was unmasked but was only permitted to perform day 1 and 7 safety visits. All other investigations, examinations, and image analyses were undertaken by masked staff.

### Intervention

Treatment-naive participants received baseline ranibizumab, 0.5 mg (Lucentis [Novartis]), then 2 additional monthly doses, then monthly pro re nata. Previously treated participants received monthly pro re nata injections after the baseline injection. Each group received 1 of 4 additional treatments shown in **Table 1**.

All participants attended day 1 and 7 after treatment, then monthly for 12 months. Treatment and visit details are detailed in eTable 1 in Supplement 2.

### Outcome Measures

The primary efficacy outcome was the mean best-corrected visual acuity (BCVA) at 3 months. An early outcome aimed to capture the treatment effect, before subsequent and perhaps unrelated changes due to neovascular AMD progression. The secondary outcome measures are shown in eTable 2 in Supplement 2.[17] Ocular imaging was analyzed centrally by masked trial retinal clinicians using Scion Image software.

The safety outcomes were all adverse events (AEs) and serious AEs (SAEs) up to month 12, coded using the Medical Dictionary for Regulatory Activities preferred terms, version 26.0 (ICH).

### Statistical Analysis

In keeping with feasibility studies, a sample size of 50 participants was deemed sufficient,[18,19] inflated to 55 to accommodate 10% attrition. The study was designed to look for trends and make preliminary estimates, and power calculations were

not undertaken. A larger control group allowed more informative estimation of the randomized effect sizes of individual interventions.

With 4 groups, there were up to 3 separate principal comparisons that could be specified, and 2 factorial comparisons were chosen based on data from all study participants: (1) TPA (groups, TPA and $C_3F_8$ + TPA) vs no TPA (groups, monotherapy control and $C_3F_8$) and (2) gas (groups, $C_3F_8$ and $C_3F_8$ + TPA) vs no gas (groups, monotherapy control and TPA).

Additional single-arm comparisons were made by comparing the pairwise comparisons of either the analysis of variance or Kruskal-Wallis test, and a univariate difference-of-differences model was used to assess for interaction. Bonferroni correction was applied to all tests of multiple comparisons. However, 95% CIs were not corrected for multiplicity.

The Shapiro-Wilk test was used to determine normality. Comparisons were made using appropriate parametric or nonparametric analysis of variance tests for continuous measures, with Fisher exact or $\chi^2$ tests for categorical outcomes. All analyses were performed on an intention-to-treat basis. Missing data were not imputed, and participants were not excluded from other analysis.

The Early Treatment Diabetic Retinopathy Study (ETDRS) letter score was converted to logMAR using 1.9 and 2.3 for count fingers and hand motions, respectively.[20] To describe changes in acuity, logMAR values were converted back to letter score, with 1 letter equaling 0.02 logMAR. Statistical analysis was performed using SPSS statistics, version 28 (IBM Corp). A 2-sided significance level of α = .05 was used.

## Results

### Baseline Characteristics

Fifty-three of 56 participants (95%; mean [SD] age, 81.5 [8.1] years; 33 female [59%]; 23 male [41%]) reached the primary end point, and 39 (70%) had preexisting neovascular AMD. Baseline characteristics are shown in **Table 2.** Recruitment averaged 3.6 participants per site per year. Fifty participants (89%) completed 12 months on trial (**Figure**). Study eyes were randomized to the following intravitreal treatments: sham injection (n = 23), $C_3F_8$ (n = 11), TPA (n = 11), or $C_3F_8$ + TPA (n = 11).

### Primary Outcome

At month 3, the factorial analysis showed a large and statistically significant logMAR BCVA difference favoring TPA (0.66) over no TPA (0.98) ($\mu_d$ = −0.32; 95% CI, −0.58 to −0.07; *P* = .02); gas vs no gas did not show a statistically significant difference (gas, 0.80 vs no gas, 0.90; $\mu_d$ = −0.11; 95% CI, −0.37 to 0.16; *P* = .43) (**Table 3**).

The comparison between individual groups did not show a statistically significant difference (control logMAR, 0.99; $C_3F_8$, 0.97 vs control, $\mu_d$ = −0.02; 95% CI, −0.48 to 0.44; TPA, 0.70 vs control, $\mu_d$ = −0.29; 95% CI, −0.79 to 0.21; $C_3F_8$ + TPA, 0.63 vs control, $\mu_d$ = −0.36; 95% CI, −0.82 to 0.11; *P* = .11) (eTable 3 in Supplement 2). There was no observed interaction between TPA and $C_3F_8$, interaction coefficient, −0.05 (95% CI, −0.58 to 0.49; *P* = .86).

**Table 1. Study Treatment Groups**

| Treatment group | Intervention |
|---|---|
| Monotherapy control | Sham intravitreal injection |
| $C_3F_8$ | Intravitreal injection of 0.3 mL of 100% $C_3F_8$ and posturing for 7 d |
| TPA | Intravitreal injection of 50 µg of alteplase in 0.05 mL (Actilyse [Boehringer Ingelheim]) |
| $C_3F_8$+TPA | Intravitreal injection of 0.3 mL of 100% $C_3F_8$ and posturing for 7 d, plus intravitreal injection of 50 µg alteplase in 0.05 mL |

Abbreviations: $C_3F_8$, perfluoropropane gas; TPA, tissue plasminogen activator.

### Secondary Outcomes

#### Visual Acuity

Mean logMAR BCVA at month 1 was significantly different comparing TPA with no TPA (0.78 vs 1.03, respectively; $\mu_d$ = −0.24; 95% CI, −0.49 to −0.02; *P* = .05) but not gas with no gas (0.80 vs 1.01; $\mu_d$ = −0.21; 95% CI, −0.46 to 0.04; *P* = .10). The superiority of TPA over no TPA was sustained at month 12 (0.60 vs 0.97; $\mu_d$ = −0.37; 95% CI, −0.68 to −0.07; *P* = .02). At month 12, the difference was not statistically significant comparing gas with no gas (0.81 vs 0.87; $\mu_d$ = −0.06; 95% CI, −0.37 to 0.25; *P* = .70).

Change in letter score from baseline to month 12 was not significantly different when comparing TPA (+10.53 letters) with no TPA (+3.76 letters; $\mu_d$ = 6.78; 95% CI, −7.76 to 21.30; *P* = .35) or gas (+11.00 letters) with no gas (+8.00 letters; $\mu_d$ = −6.0; 95% CI, −18.0 to 5.0; *P* = .26). The eFigure in Supplement 2 shows the BCVA of the 4 groups.

The percentage of participants gaining 15 letters or more at month 12 were as follows: TPA, 35% (6 of 17) vs no TPA, 39% (13 of 33; *P* = .78) and gas, 38% (8 of 21) vs no gas, 38% (11 of 29; *P* = .61).

The percentage of participants maintaining or gaining any letters at month 12 were significantly different comparing those receiving TPA 94% (16 of 17) vs no TPA 60% (20 of 33; *P* = .02). The difference was not significant between gas, 81% (17 of 21) vs no gas, 66% (19 of 29; *P* = .34). eTable 3 in Supplement 2 shows the proportions by individual group.

There was no significant difference between those participants losing 15 letters or more at month 12: TPA, 6% (1 of 17) vs no TPA, 21% (7 of 33; *P* = .24) and gas, 19% (4 of 21) vs no gas, 14% (4 of 29; *P* = .71).

#### Contrast Sensitivity

The Pelli-Robson contrast sensitivity score improved in all groups between baseline and month 12 (eTable 4 in Supplement 2). There was a significant difference in favor of TPA vs no TPA at each follow-up visit with a median month 12 score of 1.35 (IQR, 0.90-1.35) vs 0.75 (IQR, 0-1.24; *P* = .002). By month 12, there was also a significant difference favoring gas at 1.20 (IQR, 0.79-1.35) vs no gas at 0.75 (IQR, 0.26-1.35; *P* = .04). The pairwise comparisons showed a significant difference at month 12 between control at 0.68 (IQR, 0.08-1.13) and $C_3F_8$ + TPA at 1.35 (IQR, 1.01-1.39; *P* = .001) and between $C_3F_8$ at 0.98 (IQR, 0-1.35) and $C_3F_8$ + TPA at 1.35 (IQR, 1.01-1.39; *P* = .04) (eTable 5 in Supplement 2).

Table 2. Baseline Characteristics

| Baseline characteristic | Monotherapy | $C_3F_8$ | TPA | $C_3F_8$ + TPA | Overall |
|---|---|---|---|---|---|
| No. of study eyes | 23 | 11 | 11 | 11 | 56 |
| Sex, No. (%) | | | | | |
| Female | 13 (57) | 7 (64) | 8 (73) | 5 (45) | 33 (59) |
| Male | 10 (43) | 4 (36) | 3 (27) | 6 (55) | 23 (41) |
| Study eye right, No. (%) | 15 (65) | 7 (64) | 5 (46) | 5 (46) | 31 (55) |
| Study eye phakic, No. (%) | 7 (30) | 5 (46) | 6 (55) | 5 (46) | 23 (41) |
| AREDS score, mean (SD) [95% CI] | 2.38 (1.59) [1.53 to 3.22] | 1.60 (0.65) [0.79 to 2.41] | 2.10 (1.77) [−2.29 to 6.50] | 1.90 (1.82) [−0.36 to 4.16] | 2.13 (1.48) [1.56 to 2.70] |
| Age, mean (SD) [95% CI], y | 81.5 (7.09) [78.46 to 84.59] | 80.1 (4.48) [77.08 to 83.10] | 82.8 (12.92) [74.14 to 91.50] | 81.6 (7.90) [76.32 to 86.95] | 81.5 (8.14) [79.34 to 83.70] |
| Anticoagulant, No. (%) | 7 (30) | 5 (46) | 4 (36) | 4 (36) | 20 (36) |
| Antiplatelet, No. (%) | 6 (26) | 1 (9) | 5 (46) | 4 (36) | 16 (29) |
| Study eye | | | | | |
| Visual acuity, mean (SD) [95% CI], logMAR | 1.01 (0.53) [0.78 to 1.24] | 1.14 (0.48) [0.82 to 1.46] | 0.88 (0.40) [0.61 to 1.15] | 0.83 (0.42) [0.55 to 1.11] | 0.97 (0.48) [0.85 to 1.10] |
| Visual acuity of CF/HM, No. (%) | 1 (4) | 1 (9) | 0 | 0 | 0 |
| Preexisting nAMD, No. (%) | 16 (70) | 9 (82) | 8 (73) | 6 (55) | 39 (70) |
| Previous anti-VEGF, No. (%) | 7 (30) | 6 (55) | 3 (27) | 1 (9) | 17 (30) |
| No. | 23 | 9 | 10 | 11 | 53 |
| Pelli-Robson, median [IQR] | 0.45 (0 to 1.05) | 0.75 (0.60 to 0.90) | 0.75 (0.15 to 0.90) | 1.05 (0.60 to 1.28) | 0.75 (0.15 to 1.05) |
| No. | 22 | 11 | 11 | 11 | 55 |
| VFQ-25, composite score, mean (SD) [95% CI] | 60.37 (21.31) [50.92 to 69.82] | 68.61 (22.1) [51.63 to 85.60] | 58.81 (20.31) [44.29 to 73.34] | 53.43 (21.94) [38.69 to 68.17] | 60.03 (21.30) [54.10 to 65.96] |
| Imaging | | | | | |
| No. | 21 | 10 | 10 | 10 | 51 |
| Hemorrhage area, median (IQR), disc diameters | 11.4 (7.10 to 18.50) | 7.8 (3.55 to 11.38) | 15.05 (6.70 to 25.90) | 7.53 (3.08 to 12.58) | 10.10 (4.10 to 16.00) |
| No. | 21 | 9 | 10 | 10 | 50 |
| Hemorrhage greatest linear diameter, Median (IQR), µm | 6225 (5405.5 to 8681.5) | 5608 (4389 to 6422.5) | 8568.5 (4441.75 to 10695.5) | 5849 (3833.5 to 6661) | 6080.00 (4869.00 to 8260.00) |
| No. | 17 | 10 | 8 | 11 | 46 |
| OCT central retinal thickness, mean (SD) [95% CI], µm | 792.24 (312.77) [631.43 to 953.04] | 730.40 (288.02) [524.36 to 936.44] | 662.50 (204.93) [491.17 to 833.82] | 648.91 (338.04) [421.81 to 876.01] | 721.96 (295.15) [634.31 to 809.61] |

Abbreviations: AREDS, Age-Related Eye Disease study; $C_3F_8$, perfluoropropane gas; CF, count fingers; HM, hand motions; nAMD, neovascular age-related macular degeneration; OCT, optical coherence tomography; TPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; VFQ-25, National Eye Institute 25-Item Visual Function Questionnaire.

### Visual Function Questionnaire

In the factorial analysis, there was a statistically significant difference between the National Eye Institute 25-Item Visual Function Questionnaire (VFQ-25) composite score change from baseline at month 6 favoring TPA, 4.61 (IQR, −1.21 to 17.57) vs no TPA, −2.80 (IQR, −14.38 to 3.06; *P* = .02), although the difference at month 12 was no longer significant at TPA, −0.99 (IQR, −4.72 to 10.42) vs no TPA, -2.79 (IQR, −10.48 to 5.24; *P* = .33). At month 6, gas, −0.03 (IQR, −2.34 to 4.77) vs no gas, −3.12 (IQR, −15.54 to 7.58; *P* =.21) comparison was not significant and likewise at month 12, with gas, −0.90 (IQR, −3.85 to 6.68) vs no gas, −3.32 (IQR, −11.88 to 9.14; *P* = .15).

At each time point, change from baseline in the VFQ-25 composite score decreased until month 12 for the monotherapy control (−3.24; 95% CI, −13.62 to 7.14) and $C_3F_8$ (−1.89; 95% CI, −6.00 to +2.23) groups. The TPA group improved until month 6 (4.61; IQR, −5.55 to 17.57) but decreased at month 12 (−4.17; 95% CI, −14.30 to 5.96). Only the combined $C_3F_8$ + TPA group had an improvement at month 12 (8.27; 95% CI, −9.72 to 26.26). The difference at each time point was not statistically significant across the 4 groups (eTables 4 and 5 in Supplement 2).

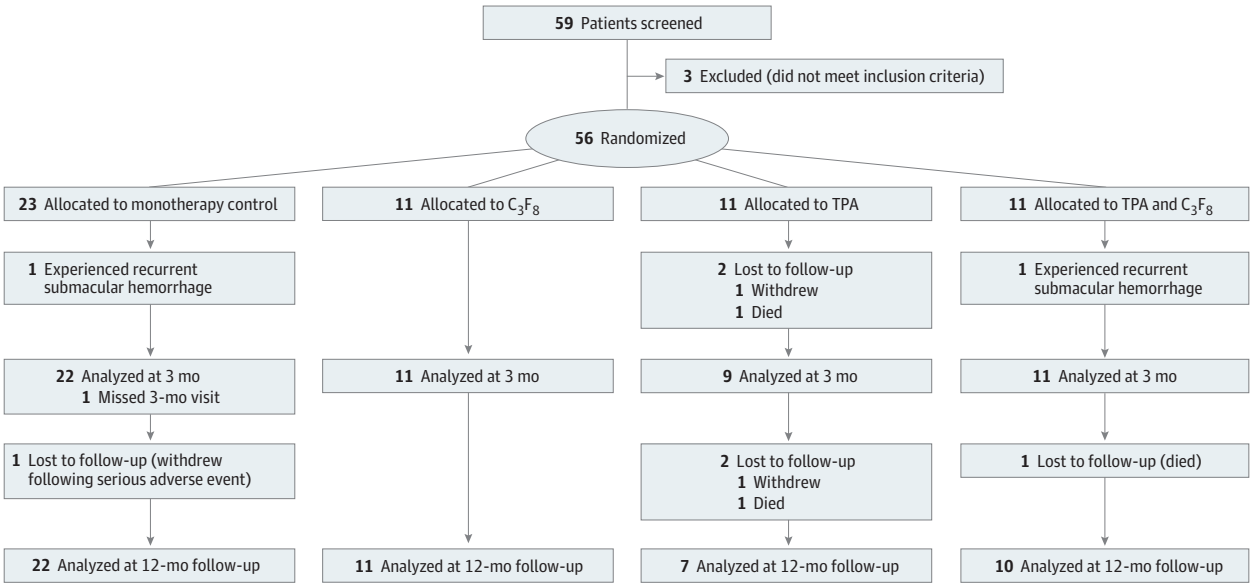
### Lens Status

At baseline 59% of study eyes (33 of 56) were phakic. Three participants in the monotherapy control group and 1 in the $C_3F_8$ + TPA group underwent cataract surgery (eTables 4 and 5 in Supplement 2).

### Ranibizumab Retreatment

In both the TPA and gas factorial comparisons, there was no significant difference in the mean number of injections re-

Figure. Trial Profile




Consolidated Standards of Reporting Trials diagram detailing participant allocation and exclusions. $C_3F_8$, indicates perfluoropropane gas; TPA, tissue plasminogen activator.

ceived (TPA, 4.62; 95% CI, 3.41-5.83 vs no TPA, 5.23; 95% CI, 4.13-6.32; *P* = .25; gas, 5.15; 95% CI, 3.98-6.32 vs no gas, 4.88; 95% CI, 3.77-5.98; *P* = .37) (eTables 6 and 7 in Supplement 2).

### Macular Hemorrhage and Fibrosis

At month 1 subfoveal blood was present in 51.1% of study eyes (31 of 42): only 55.6% of individuals (10 of 18) receiving TPA, but 87.5% of those (21 of 24) who did not (*P* = .03). There was no significant difference comparing the gas (73.3% [11 of 15]) and no gas (74.1% [20 of 27]) groups (*P* = .62) (eTable 8 in Supplement 2).

There were no statistically significant differences between the groups comparing hemorrhage size or greatest linear diameter at months 1, 3, and 6 (eTable 9 in Supplement 2).

By month 12, the median central retinal thickness decreased in all groups (eTable 9 in Supplement 2). There was no significant difference comparing TPA (395.0 µm; IQR, 295-496 µm) and no TPA (403.0 µm; IQR, 321-535 µm; *P* = .56) or gas (395.0 µm; IQR, 305-484 µm) and no gas (398.0 µm; IQR, 285-527 µm; *P* = .82).

Month 12 angiography showed fibrosis in 51.2% of study eyes (22 of 43). Eyes receiving TPA had less fibrosis (31.3% [5 of 16]) than those that did not (63.0% [17 of 27]; *P* =.06), but the result was not quite significant. There was no significant difference comparing gas (58.8% [8 of 17 eyes]) and no gas (53.8% [14 of 26 eyes; *P* = .76) (eTable 9 in Supplement 2).

### Safety

The AEs are listed in eTables 10, 11, 12, and 14 in Supplement 2. Of 21 study eye AEs, 6 were SAEs. Raised intraocular pressure occurred in 2 of 22 eyes (9.1%) receiving $C_3F_8$, with both responding to topical medication alone.

There were 2 SMH recurrences, one each in the monotherapy control and combined $C_3F_8$ + TPA groups (eTable 13 in Supplement 2). The participant in the control group was treated with a pars plana vitrectomy with subretinal TPA and anti-VEGF to manage the recurrence. The participant in the combined $C_3F_8$ + TPA group received repeat intravitreal TPA and gas. This patient then subsequently exited the study consequent to a fatal hemorrhagic stroke. Both participants had concomitant antiplatelet medication but did not receive anticoagulant treatment. One further participant died of stroke, and a third died after a mechanical fall and long hospitalization. Three further patients withdrew from the study, one due to nonocular AE and hospitalization, one was lost to follow-up, and the third emigrated from the country.

## Discussion

Although the TAPAS study was primarily designed to inform a future definitive trial and was not powered to detect a statistically significant difference between groups, the main factorial analysis showed a statistically significant benefit in the primary outcome in the groups receiving intravitreal TPA, with a large and clinically meaningful effect size (+16 ETDRS letters; *P* =.02). The combined gas groups did not show as much benefit (+5 letters; *P* = .43). The contrast sensitivity, VFQ-25, fibrosis, and SMH size analyses also showed benefit from TPA at various time points, benefits that were much less evident or absent with gas. These findings were unexpected, suggesting TPA and ranibizumab drive vision gains, rather than gas. There was also no evidence supporting an interaction between TPA and gas.

Table 3. Visual Acuity Outcomes for Factorial Analysis

| Acuity outcome | TPA | No TPA | Mean difference (95% CI) | *P* value | Unadjusted odds ratio | Gas | No gas | Mean difference (95% CI) | *P* value | Unadjusted odds ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | 22 | 33 | NA | NA | NA | 22 | 33 | NA | NA | NA |
| Month 1 visual acuity, mean (SD) [95% CI], logMAR | 0.78 (0.39) | 1.03 (0.48) | −0.24 (−0.49 to 0.02) | .05 | NA | 0.80 (0.40) | 1.01 (0.48) | −0.21 (−0.46 to 0.04) | .10 | NA |
| No. | 20 | 33 | NA | NA | NA | 22 | 31 | NA | NA | NA |
| Primary outcome | | | | | | | | | | |
| Month 3 visual acuity, mean (SD) [95% CI], logMAR | 0.66 (0.37) | 0.98 (0.49) | −0.32 (−0.58 to −0.07) | .02 | NA | 0.80 (0.40) | 0.90 (0.52) | −0.11 (−0.37 to 0.16) | .43 | NA |
| No. | 17 | 33 | NA | NA | NA | 20 | 30 | NA | NA | NA |
| Month 6 visual acuity, mean (SD) [95% CI], logMAR | 0.67 (0.39) | 1.03 (0.54) | −0.37 (−0.66 to −0.07) | .02 | NA | 0.85 (0.39) | 0.95 (0.59) | −0.11 (−0.41 to 0.20) | .48 | NA |
| No. | 17 | 33 | NA | NA | NA | 21 | 29 | NA | NA | NA |
| Month 12 visual acuity, mean (SD) [95% CI], logMAR | 0.60 (0.41) | 0.97 (0.55) | −0.37 (−0.68 to −0.07) | .02 | NA | 0.81 (0.49) | 0.87 (0.57) | −0.06 (−0.37 to 0.25) | .70 | NA |
| No. | 17 | 33 | NA | NA | NA | 21 | 29 | NA | NA | NA |
| Month 12 change in letter score, mean (SD) | 10.53 (9.85) | 3.76 (28.81) | 6.78 (−7.76 to 21.3) | .35 | NA | 11.00 [5.00 to 22.5][a] | 8.00 [−6.00 to 16.5][a] | −6.0 [−18.0 to 5.0][b] | .26 | NA |
| Month 12 ≥15 letter gain, No. (%) | 6 (35.3) | 13 (39.4) | NA | .78 | 0.84 (0.25 to 2.83) | 8 (38.1) | 11 (37.9) | NA | .99 | 1.01 (0.32 to 3.20) |
| Month 12 0 letter gain, No. (%) | 16 (94.1) | 20 (60.6) | NA | .02 | 10.40 (1.23 to 88.2) | 17 (81.0) | 19 (65.5) | NA | .34 | 2.24 (0.59 to 8.47) |
| Month 12 ≥15 letter loss, No. (%) | 1 (5.9) | 7 (21.2) | NA | .24 | 0.23 (0.03 to 2.07) | 4 (19.0) | 4 (13.8) | NA | .71 | 1.47 (0.32 to 6.70) |

Abbreviations: $C_3F_8$, perfluoropropane gas; NA, not applicable; TPA, tissue plasminogen activator.

[a] Median (IQR).

[b] Hodges-Lehman median difference.

Safety was acceptable in all groups. Two participants receiving intravitreal TPA experienced a stroke, but many participants had risk factors for stroke, which is not uncommon in this age group. Also, the half-life of intravitreal TPA is likely less than 12 hours, yet the strokes occurred 2 and 3 months after treatment, making a biological link less plausible.[21] The incidence of cataract was similar between the groups. Only 2 participants in the gas groups experienced raised intraocular pressure, and these resolved uneventfully with eye drops.

At 12 months, all groups had improved mean BCVA. These results are far better than those reported before the introduction of anti-VEGF therapy, with patients typically losing 3.2 lines at 12 months.[22] Although individual groups were not significantly different (unlike the main factorial comparison), for the primary outcome, the anti-VEGF monotherapy group had a logMAR BCVA of 0.99, very similar to the gas group (0.97); by comparison, the TPA group had a materially better outcome (0.70) that was slightly better yet with the addition of gas (0.63). The BCVA gain was similar to other studies investigating anti-VEGF therapy combined with gas,[23-27] or both gas and TPA,[9,28-30] although not quite as good as the recent Surgery, Tissue Plasminogen Activator, Antiangiogenic Agents and Age-Related Macular Degeneration (STAR) RCT, which reported a mean improvement at 6 months of 15.4 ETDRS letters in 37 participants receiving intravitreal ranibizumab, TPA, and sulfur hexafluoride gas.[16] Although a small retrospective case series investigated vitrectomy, subretinal TPA, and intravitreal anti-VEGF therapy,[31] to our knowledge, our study was the first RCT investigating intravitreal TPA without gas.

At all time points, the TPA groups had better contrast sensitivity outcomes than the groups without TPA, whereas gas was only significantly better than no gas at month 12. The VFQ-25 composite score showed a mean decrease at the month 12 time point for all groups except the combined $C_3F_8$ + TPA group. Most VFQ-25 statistical comparisons were nonsignificant, although at 6 months, the groups receiving TPA had a significantly better gain than those that did not. Considering the VFQ-25 and contrast sensitivity results in toto suggests that TPA produces more convincing benefit than gas, but due to the small size, it is hard to exclude additional benefit from adding gas to TPA, as suggested by the month 12 VFQ-25 result.

Although large SMH is a rare condition and recruitment was predictably slow, it was feasible to recruit 56 participants across 4 sites, with good retention at 3 and 12 months. This trial deliberately included a large number of outcome measures, to assess which are the most useful for a definitive study. The mean

month 3 BCVA was a discriminating and deliverable primary outcome. Despite a considerable burden of comorbidity in many participants, the retention rates at month 12 suggest a later outcome may also be deliverable and may be informative for patients and clinicians. Change in BCVA is often a helpful metric, but due to the often poor presenting BCVA, measurements may be unreliable, and therefore, final BCVA may be better. Contrast sensitivity and VFQ-25 showed meaningful differences between groups, as did the structural outcomes.

These results might argue for a future comparison of anti-VEGF monotherapy, representing the licensed standard of care for neovascular AMD, vs anti-VEGF and TPA, as the gains from TPA were similar to those of TPA and gas, and this avoids the burden of face down positioning. An alternative is to compare anti-VEGF monotherapy and combined anti-VEGF, TPA, and gas. The rationale of adding gas is that the TAPAS study was not large enough to exclude a benefit from gas. A few metrics suggest it helps, and gas had acceptable safety and clinicians may be more receptive to trial participation.

Consistent with the latter option, the Vitrectomy, Subretinal Tissue Plasminogen Activator, and Intravitreal Gas for Submacular Hemorrhage Secondary to Exudative Age-Related Macular Degeneration (TIGER) study is an ongoing 210-participant, noncommercial RCT comparing aflibercept monotherapy against aflibercept, TPA, and gas tamponade, albeit with the TPA delivered transretinally via vitrectomy.[32]

### Strengths and Limitations

The TAPAS study was the second largest RCT of SMH, a rare disease, and with longer follow-up time than prior RCTs of SMH. Weaknesses include the higher dropout rate in the TPA group by month 12 (4 of 11 participants). However, this was not due to ocular SAEs and may be due to chance, as the gas and TPA group had good retention. Although the study masked the delivery of TPA by sham injection, it was not possible to mask the delivery of gas, which might introduce performance bias for functional tests, but assessors were all masked and used protocol standardized methodology. It is not known if the same results would occur with other anti-VEGF agents, but given that most have a similar mode of action, this might be expected. Baseline differences between groups might alter the likelihood of vision improvement.

## Conclusions

In summary, in this factorial RCT, results suggest that TPA may increase the chance of visual acuity gain when added to ranibizumab therapy for neovascular AMD in eyes with SMH. Therefore, a definitive trial against anti-VEGF monotherapy appears justified and deliverable, with potential visual acuity benefits appearing to be driven largely by TPA in combination with anti-VEGF.


### ARTICLE INFORMATION

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**Correction:** This article was corrected on December 19, 2024, to remove Ms Saleh from statistical analysis in the author contributions section, add a conflict of interest disclosure for Ms Saleh, and correct the typography of Dr Murphy's degree in the byline.

**Author Contributions:** Prof Jackson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
*Concept and design:* Steel, Jackson.
*Acquisition, analysis, or interpretation of data:* All authors.
*Drafting of the manuscript:* Murphy.
*Critical review of the manuscript for important intellectual content:* Murphy, Saleh, Ayis, Cheema, Mehta, Steel, Membrey, Costen, Jackson.
*Statistical analysis:* Murphy, Ayis, Steel.
*Obtained funding:* Jackson.
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**Conflict of Interest Disclosures:** Dr Murphy reported receiving Roche site payment to institution for commercial clinical trial in AMD and Opthea site payment to institution for commercial clinical trial in AMD outside the submitted work. Ms Saleh reported being an employee of National Health Service (NHS), which received payment for commercial clinical trials. Prof Steel reported receiving grants from Alcon, Roche, DORC, Bayer, Gyroscope, Boehringer Ingelheim, and BVI and personal fees from Alcon, Roche, DORC, BVI, Gyroscope, Eyepoint, and Complement Therapeutics outside the submitted work. Prof Jackson reported receiving consultant/advisor fees from the following (none related to tissue plasminogen activator): 2CTech, Alcon, Dutch Ophthalmic Research Centre, iLumen, Opthea, Outlook Therapeutics, Oxurion, and Regeneron and conference support from Roche; Prof Jackson's NHS employer receives site fees for patients enrolled on commercial retinal trials of AMD and other conditions. Free provision of AMD drug by Bayer for noncommercial trial sponsored by Prof Jackson' employer, and free use of treatment or diagnostic device from Zeiss and LKC respectively, for noncommercial studies sponsored by Prof Jackson's employer. No other disclosures were reported.

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Invited Commentary

# Can We Do Better for Submacular Hemorrhage in Neovascular AMD?

Christina Y. Weng, MD, MBA

**The topic of this commentary** hits close to home, as a patient of mine recently developed a submacular hemorrhage (SMH). This is a dreaded condition, not only because of the negative impact it can have on visual prognosis but because of its acute and unpredictable presentation in an era where remarkably effective treatments for neovascular age-related macular degeneration (AMD) otherwise exist. Anti-vascular endothelial growth factor (anti-VEGF) intravitreal injections have contributed to the growing number of patients with neovascular AMD living with excellent visual acuity (VA). But SMH can rob patients, like mine, of their vision. Thus, the quest to identify optimal treatment continues, propelled by studies like the one published by Murphy et al[1] in this issue of *JAMA Ophthalmology*.

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In a factorial randomized clinical trial of 56 patients, Murphy and colleagues[1] assess whether intravitreal tissue plasminogen activator (TPA) or perfluoropropane gas ($C_3F_8$) improves VA or promotes resolution of SMH secondary to neovascular AMD in eyes treated with ranibizumab.[1] Study eyes with fovea-involving SMH seen within 14 days of onset received ranibizumab and were then randomized to sham injection, $C_3F_8$, TPA, or $C_3F_8$ + TPA. All eyes received