Summary of findings tables, grading of the evidence and detailed conclusions of evidence female fertility preservation

Who should be informed about potential infertility risk?

1. **What is the patient and/or parents/caregivers/partners reported desire and satisfaction with the information about treatment-related fertility risks, fertility preservation and alternative family planning options provided to cancer patients diagnosed before age 25 years?**

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.1. Satisfaction with information reported by patients (n=2 studies)	Benedict 2016	179 young adult female cancer survivors	23.6 years (0 -35 years) Subgroup: 23.4 years (0-34 years)	Survey	*Dissatisfaction with content of fertility preservation discussion* Female cancer patients reported unmet information needs regarding fertility risks (58-60%), options to assess and preserve fertility (51-62%), and options for alternative family planning (43%)
	Yeomanson 2013	290 current and former cancer patients attending a cancer trust conference: 150 delegates in 2004 140 delegates in 2011	Median age at start of treatment: 2004: Boys: 16 years (13-22) Girls: 15 years (13-21) 2011: Boys: 16 years (13-22) Girls: 17 years (13-22)	Structured and standardized survey	*Dissatisfaction with content of fertility preservation discussion* 35.8% of male patients and 50% of female patients were unsatisfied with the content of fertility preservation discussion
GRADE Assessment: Methodological limitations: Coherence: Adequacy of data: Relevance:		Some methodological limitations in 2/2 No concerns on coherence No concerns on adequacy of data (2 studies; 469 study participants) No concerns on relevance (>85% cancer patients in 2/2)			
Overall assessment of confidence in findings:		**MODERATE confidence in the evidence***			
Conclusion:		Most patients (pre- and postpubertal) are not satisfied with the content of fertility-related discussions with their (pediatric) oncology health healthcare providers , *(2 surveys; 469 study participants)* especially, about information received on fertility risks, options to preserve fertility and alternative family planning *(1 survey; 179 study participants)*			

Abbreviations: NM, not mentioned; NA, not applicable

* Adapted methodology from GRADE and Confidence in the Evidence from Reviews of Qualitative Research (GRADE-CERQual).

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.2. Desire for information reported by patients and parents (n=1 study)	Gupta 2013	243 cancer patients receiving treatment, or within 5 years of completion of treatment	Age at diagnosis: NM Age at study: median 28 years (17-35 years)	Survey Adapted existing survey to use Likert Scale of importance (1-10) Piloted study with 10 patients and 10 healthcare providers	*Desire for information in fertility preservation discussion* Patients reported information about the effects of cancer treatment on fertility and fertility preservation before cancer treatment as very important (median scores of 9 and 10 in scale 1-10) Female patients rated information on fertility preservation methods (p=0.004) and risk of infertility (p=0.033) as more important than did male patients
GRADE Assessment: Methodological limitations: Some methodological limitations Coherence: No concerns on coherence Adequacy of data: Some concerns on adequacy of data: 1 study investigating desire of information in fertility preservation discussion; (1 study; 243 study participants) Relevance: No concerns on relevance (all cancer patients)					
Overall assessment of confidence in findings: **LOW confidence in the evidence*** **Conclusion:** Post-pubertal patients have a high desire for information about the effects of cancer treatment on fertility (median score 9) and options for fertility preservation (median score 10) (scale 1-10, includes male and female) *(1 survey; 243 study participants)*					

Abbreviations: NM, not mentioned; NA, not applicable

* Adapted methodology from GRADE and Confidence in the Evidence from Reviews of Qualitative Research (GRADE-CERQual).

Outcome	Study	Participants	Age at patients' diagnosis	Method	Summary of findings
1.3. Desire for information reported by healthcare providers (n=1 study)	Quinn 2009a	24 pediatric oncologists	NM	Semistructured in-depth interviews	*Desire for information about fertility preservation (according to healthcare professionals)* 50% of pediatric oncologists reported that parents and patients want fertility preservation information, but parents and patients are either too embarrassed to discuss it or do not know how to begin a discussion
GRADE Assessment:					

Methodological limitations:	Some methodological limitations in 1/1
Coherence:	NA (1 study only)
Adequacy of data:	Important concerns on adequacy of data (1 study; 24 study participants)
Relevance:	Important concerns on relevance (pediatric oncologists reporting on behalf of patients and parents)
Overall assessment of confidence in findings:	**VERY LOW confidence in the evidence***
Conclusion:	Some patients and their parents desire information about fertility preservation but experience difficulties initiating discussions on this topic *(1 semistructured in-depth interview study; 24 study participants*)

Abbreviations: NM, not mentioned; NA, not applicable

***** Adapted methodology from GRADE and Confidence in the Evidence from Reviews of Qualitative Research (GRADE-CERQual).

Who should be counselled about fertility preservation?

1. What is the risk of POI in female cancer patients diagnosed before age 25 years who will be treated with alkylating agents?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.1 Risk POI after alkylating agents (any type) (n=14 studies)	Byrne 1992	1,048 CCS vs. 1,596 siblings	>19 yr after cancer diagnosis	Alkylating agents: at least 11.1%; Radiotherapy to ovaries: 7.5%	123/954 (12.9%) amenorrhoea after study entry	*Relative risk (95% CI) for amenorrhea* Alkylating agents yes vs. no (controls): RR 9.17 (2.67-31.49)	SB: unclear AB: low risk DB: unclear CF: high risk
	Chiarelli 1999	719 CCS vs. 162 CCS with non-sterilizing surgery	5-30 yr after diagnosis	Alkylating agents: at least 20.1%; Radiotherapy to ovaries: 21.4%	63/719 (8.8%) amenorrhoea after treatment	*Risk ratio (95% CI) for amenorrhea* Alkylating agents vs. non-sterilizing surgery: RR 0.77 (0.30-1.97); Alkylating agent score vs. non-sterilizing surgery: 1-13: RR 1.13 (0.41-3.09) 14-21: RR 1.90 (0.52-6.92) ≥21: RR 3.08 (1.15-8.21)	SB: high risk AB: unclear DB: unclear CF: low risk
	Chemaitilly 2006*	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Odds ratio (95% CI) for amenorrhea age at diagnosis 0-12 yr* Cyclophosphamide yes vs. no: OR 1.2 (0.7-2.1); Procarbazine yes vs. no: OR 3.2 (1.3-7.3); *Odds ratio (95% CI) for*	SB: high risk AB: low risk DB: unclear CF: low risk

						amenorrhea age at diagnosis 13-20 yr Cyclophosphamide yes vs. no: OR 4.9 (2.8-9.2); Procarbazine yes vs. no: OR 2.6 (1.4-4.7)	
	Sklar 2006*	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Risk ratio (95% CI) for (non-surgical) amenorrhea* Alkylating agent score 1-2 vs. 0: RR 2.3 (1.08-4.90); Alkylating agent score 3 vs. 0: RR 5.78 (2.9-11.55)	SB: high risk AB: low risk DB: unclear CF: low risk
	Laverdiere 2005*	32 neuroblastoma survivors	Median 7.06 yr (range 1.9-25.5) after cancer diagnosis	Cyclophosphamide: 100%; Radiotherapy to ovaries: ±82.5%	13/32 (41%) ovarian failure (not specified)	*Odds ratio (95% CI) for ovarian failure (not specified)* Cyclophosphamide ≥7.4 g vs. <7.4 g: OR 9.62 (1.4-67.2)	SB: unclear AB: low risk DB: unclear CF: low risk
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Risk for amenorrhea* Cyclophosphamide was not significantly associated (no effect measure reported); *Univariate odds ratio (95% CI) for amenorrhea* Alkylating agent score >3 vs. 0: OR 12 (2.0-71.0); Alkylating agent score 2 vs. 0: OR 2.0 (1.0-33.2); Cumulative cyclophosphamide dose >5 g vs. <5 g: OR 7.1 (1.5-34.0)	SB: high risk AB: low risk DB: unclear CF: low risk
	Gracia 2012	71 CCS	>1 yr after cancer treatment	Alkylating agents: 88.7%; Radiotherapy to ovaries: 18.3%	NM 49/71 (69.0%) regular cycles	*Geometric mean FSH* Alkylating agent score: β 0.91, p=0.016 (Each unit increase in alkylator score, geometric mean FSH values increased by 0.91 IU/L)	SB: unclear AB: low risk DB: unclear CF: low risk
	Borgman-Staudt 2012	138 childhood and adolescent HSCT survivors	Median 6 yr after HSCT (range 3-12)	Any alkylating agent: 71% Busulfan: 29%; Cyclophosphamide: 48%; TBI: 39%	111/133 (83%) impaired fertility (amenorrhoea, hormone substitution,	*Odds ratio (95% CI) for impaired fertility* Busulfan yes vs. no: OR 47.4 (5.4-418.1); Cyclophosphamide not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk

					↑ FSH/ or ↓ estradiol)		
	Thomas-Teinturier 2013*	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Cyclophosphamide: 40.2%; Procarbazine: 7.2%; Lomustine: 2.1%; Mechlorethamine: 5.7%; Ifosfamide: 3.1%; Dacarbazine: 2.5%; Carmustine: 2.0%; Melphalan: 1.3%; Thiotepa: 0.1%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Procarbazine dose per g/m^2: RR 2.5 (1.4-5.8); Cyclophosphamide dose per g/m^2: RR 1.3 (1.0-2.1); Melphalan yes vs. no: RR 15.2 (3.2-52.7); Alkylating agents before pubertal period vs. none: RR 2.8 (1.2-6.5); Alkylating agents during pubertal period vs. none: RR 14.8 (4.2-52.8); Alkylating agents after menses vs. none: RR 7.6 (3.0-19.1); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr* Melphalan yes vs. no: RR 32.0 (2.0-530.0); Cumulative cyclophosphamide dose per g/m^2: RR 1.1 (1.02-1.3)	SB: high risk AB: low risk DB: unclear CF: low risk
	Bresters 2014	109 childhood HSCT survivors	Median 7.2 yr afer HSCT (>2 years after HSCT)	Any alkylating agent: 100%; Cyclophosphamide: 90.8%; Busulfan: 31.2%; Melphalan: 20.2%; Ifosfamide: 1.8%; Treosulfan: 7.3%; Tiothepa: 2.8%; Etoposide: 4.6%; Radiotherapy to ovaries: 53.2%	61/109 (56%) ovarian insufficiency (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development after age 12 yr or in post-pubertal amenorrhea)	*Relative risk (95% CI) for ovarian insufficiency* Chemotherapy with vs. without busulfan: RR 2.98 (0.99-9.03), p=0.05	SB: low risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2015*	108 CCS	>3 yr after cancer treatment	Any alkylating agent: 100%;	8/108 (7.6%) altered	*Mean FSH* Procarbazine dose: β 0.012,	SB: high risk AB: low risk

				Cyclophosphamide: 67.6%; Ifosfamide: 31.4 %; Procarbazine: 21.9%; Radiotherapy to ovaries: 17.6%	ovarian function (↑ FSH, ↓ AMH and amenorrhoea)	p<0.001; High-dose alkylating agents: β 0.197, p=0.09 (Each unit increase in procarbazine dose, mean FSH values increased by 0.012 IU/L)	DB: unclear CF: low risk
	Chemaitilly 2017	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* CED <8000 vs. 0 mg/m²: HR 1.55 (0.77-3.11); CED 8000-11999 vs. 0 mg/m²: HR 2.77 (1.18-6.51); CED 12000-19999 vs. 0 mg/m²: HR 3.90 (1.80-8.43); CED ≥20000 vs. 0 mg/m²: HR 4.13 (1.63-10.50); Alkylating agents only vs. no alkylating agents nor ovarian radiotherapy: HR 2.98 (0.63-14.06)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Procarbazine dose <4000 mg/m² vs. 0: OR 3.07 (0.76-12.43); Procarbazine dose ≥4000 mg/m² vs. 0: OR 8.96 (5.02-16.00); Cyclophosphamide equivalence dose <6000 mg/m² vs. 0: OR 0.80 (CI 0.32-2.01); CED ≥6000 mg/m² vs. 0: OR 3.47 (2.08-5.78); CED without procarbazine <6000 mg/m² vs. 0: OR 0.71 (0.28-1.83); CED without procarbazine ≥6000 mg/m² vs. 0: OR 1.07 (0.50-2.28) CED without procarbazine ≤2000 mg/m²: 2/200 CCS with NSPM	SB: high risk AB: low risk DB: unclear CF: low risk
	Fernandez Pineda 2018*	90 childhood Hodgkin lymphoma survivors	>10 yr after cancer diagnosis	Alkylating agents: 97%; Radiotherapy to ovaries: 100%	Events NM (premature ovarian insufficiency defined as	*Hazard ratio (95% CI) for premature ovarian insufficiency* CED 8,001-12,000 vs. ≤8,000 mg/m²: HR 3.3 (0.7-16.0);	SB: low risk AB: low risk DB: unclear CF: low risk

				absence of menses 5 years post cancer diagnosis or loss of spontaneous menses prior to 40 years of age with laboratory or historic evidence of primary (ovarian) origin)	CED 12,001-20,000 vs. ≤8,000 mg/m²: HR 11.2 (3.4-36.8); CED >20,000 vs. ≤8,000 mg/m²: HR 36.9 (5.2-260.5)	
GRADE assessment:						
Study design:	+4	Retrospective cohort studies				
Study limitations:	-1	Limitations: Selection bias low in 2/14, high in 8/14, unclear in 4/14; Attrition bias low in 13/14, unclear in 1/14; Detection bias unclear in 14/14; Confounding low in 13/14, high in 1/14				
Consistency:	0	No important inconsistency, all show effect of alkylating agents (1 study non-significant result)				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	0	No important imprecision, high total number of events and narrow confidence intervals				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect in all studies				
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊕⊕ HIGH				
Conclusion:		Increased risk of POI after alkylating agents vs. no alkylating agents in female cancer survivors diagnosed before age 25 years. (13 studies significant effect, 1 study non-significant effect; 14,035 participants; 1005 events; 13 multivariable analyses)				

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CED, cyclophosphamide equivalence dose; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.
* Overlap in included patients in studies of Laverdiere 2005, Laverdiere 2009, Chemaitily 2006, Sklar 2006 and Levine 2018; Chemaitily 2017 and Fernandez-Pineda 20118; and Thomas-Teinturier 2013 and 2015.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.2 Risk POI after higher vs. lower alkylating agent dose (any type)	Chiarelli 1999	719 CCS vs. 162 CCS with non-sterilizing surgery	5-30 yr after diagnosis	Alkylating agents: at least 20.1%; Radiotherapy to ovaries: 21.4%	63/719 (8.8%) amenorrhoea after treatment	*Risk ratio (95% CI) for amenorrhea* Alkylating agent score vs. non-sterilizing surgery: 1-13: RR 1.13 (0.41-3.09) 14-21: RR 1.90 (0.52-6.92)	SB: high risk AB: unclear DB: unclear CF: low risk

(n=10 studies)						≥21: RR 3.08 (1.15-8.21)	
	Sklar 2006*	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Risk ratio (95% CI) for (non-surgical) amenorrhea* Alkylating agent score 1-2 vs. 0: RR 2.3 (1.08-4.90); Alkylating agent score 3 vs. 0: RR 5.78 (2.9-11.55)	SB: high risk AB: low risk DB: unclear CF: low risk
	Laverdiere 2005*	32 neuroblastoma survivors	Median 7.06 yr (range 1.9-25.5) after cancer diagnosis	Cyclophosphamide: 100%; Radiotherapy to ovaries: ±82.5%	13/32 (41%) ovarian failure (not specified)	*Odds ratio (95% CI) for ovarian failure (not specified)* Cyclophosphamide ≥7.4 g vs. <7.4 g: OR 9.62 (1.4-67.2)	SB: unclear AB: low risk DB: unclear CF: low risk
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Univariate odds ratio (95% CI) for amenorrhea* Alkylating agent score >3 vs. 0: OR 12 (2.0-71.0); Alkylating agent score 2 vs. 0: OR 2.0 (1.0-33.2); Cumulative cyclophosphamide dose >5 g vs. <5 g: OR 7.1 (1.5-34.0)	SB: high risk AB: low risk DB: unclear CF: high risk
	Gracia 2012	71 CCS	>1 yr after cancer treatment	Alkylating agents: 88.7%; Radiotherapy to ovaries: 18.3%	NM 49 (69.0%) regular cycles	*Geometric mean FSH* Alkylating agent score: β 0.91 mIU/ml, p=0.016 (Each unit increase in alkylator score, geometric mean FSH values increased by 0.91 mIU/mL)	SB: unclear AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Cyclophosphamide: 40.2%; Procarbazine: 7.2%; Lomustine: 2.1%; Mechlorethamine: 5.7%; Ifosfamide: 3.1%; Dacarbazine: 2.5%; Carmustine: 2.0%; Melphalan: 1.3%; Thiotepa: 0.1%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Procarbazine dose per g/m^2: RR 2.5 (1.4-5.8); Cyclophosphamide dose per g/m^2: RR 1.3 (1.0-2.1); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr* Cumulative cyclophosphamide dose per g/m^2: RR 1.1 (1.02-1.3)	SB: high risk AB: low risk DB: unclear CF: low risk

	Thomas-Teinturier 2015	108 CCS	>3 yr after cancer treatment	Any alkylating agent: 100%; Cyclophosphamide: 67.6%; Ifosfamide: 31.4 %; Procarbazine: 21.9%; Radiotherapy to ovaries: 17.6%	8/108 (7.6%) altered ovarian function (↑ FSH, ↓ AMH and amenorrhoea)	*Mean FSH* Procarbazine dose: β 0.012, p<0.001; High-dose alkylating agents: β 0.197, p=0.09 (Each unit increase in procarbazine dose, mean FSH values increased by 0.012 IU/L)	SB: high risk AB: low risk DB: unclear CF: low risk
	Chemaitilly 2017*	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhoea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* CED <8000 vs. 0 mg/m^2: HR 1.55 (0.77-3.11); CED 8000-11999 vs. 0 mg/m^2: HR 2.77 (1.18-6.51); CED 12000-19999 vs. 0 mg/m^2: HR 3.90 (1.80-8.43); CED ≥20000 vs. 0 mg/m^2: HR 4.13 (1.63-10.50)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Procarbazine dose <4000 mg/m^2 vs. 0: OR 3.07 (0.76-12.43); Procarbazine dose ≥4000 mg/m^2 vs. 0: OR 8.96 (5.02-16.00); CED <6000 mg/m^2 vs. 0: OR 0.80 (CI 0.32-2.01); CED ≥6000 mg/m^2 vs. 0: OR 3.47 (2.08-5.78); CED without procarbazine <6000 mg/m^2 vs. 0: OR 0.71 (0.28-1.83); CED without procarbazine ≥6000 mg/m^2 vs. 0: OR 1.07 (0.50-2.28) CED without procarbazine ≤2000 mg/m^2: 2/200 CCS with NSPM	SB: high risk AB: low risk DB: unclear CF: low risk
	Fernandez Pineda 2018*	90 childhood Hodgkin lymphoma survivors	>10 yr after cancer diagnosis	Alkylating agents: 97%; Radiotherapy to ovaries: 100%	Events NM (premature ovarian insufficiency defined as absence of menses 5 years post cancer	*Hazard ratio (95% CI) for premature ovarian insufficiency* CED 8,001-12,000 vs. ≤8,000 mg/m^2: HR 3.3 (0.7-16.0); CED 12,001-20,000 vs. ≤8,000 mg/m^2: HR 11.2 (3.4-36.8);	SB: low risk AB: low risk DB: unclear CF: low risk

				diagnosis or loss of spontaneous menses prior to 40 years of age with laboratory or historic evidence of primary (ovarian) origin)	CED >20,000 vs. ≤8,000 mg/m²: HR 36.9 (5.2-260.5)	
GRADE assessment:						
Study design:	+4	Retrospective cohort studies				
Study limitations:	-1	Limitations: Selection bias low in 1/10, high in 7/10, unclear in 2/10; Attrition bias low in 9/10, unclear in 1/10; Detection bias unclear in 10/10; Confounding low in 9/10, high in 1/10				
Consistency:	0	No important inconsistency, all show that higher doses of alkylating agents are associated with higher risk				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	0	No important imprecision, large sample size and high total number of events				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect in all studies				
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊕⊕ HIGH				
Conclusion:		Increased risk of POI after increasing doses of alkylating agents in female cancer survivors diagnosed before age 25 years. (9 studies significant effect; 9,152 participants; 495 events; 9 multivariable analyses)				

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CED, cyclophosphamide equivalence dose; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; NM, not mentioned; SB, selection bias; yr, year.

* Overlap in included patients in studies of Laverdiere 2005, Laverdiere 2009, Sklar 2006 and Levine 2018; Chemaitilly 2017 and Fernandez-Pineda 2018; and Thomas-Teinturier 2013 and 2015.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.3 Risk POI after cyclophosphamide (n=6 studies)	Chemaitilly 2006	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Odds ratio (95% CI) for amenorrhea age at diagnosis 0-12 yr* Cyclophosphamide yes vs. no: OR 1.2 (0.7-2.1) *Odds ratio (95% CI) for amenorrhea age at diagnosis 13-20 yr* Cyclophosphamide yes vs. no: OR 4.9 (2.8-9.2)	SB: high risk AB: low risk DB: unclear CF: low risk

	Laverdiere 2005*	32 neuroblastoma survivors	Median 7.06 yr (range 1.9-25.5) after cancer diagnosis	Cyclophosphamide: 100%; Radiotherapy to ovaries: ±82.5%	13/32 (41%) ovarian failure (not specified)	*Odds ratio (95% CI) for ovarian failure (not specified)* Cyclophosphamide ≥7.4 g vs. <7.4 g: OR 9.62 (1.4-67.2)	SB: unclear AB: low risk DB: unclear CF: low risk
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Risk for amenorrhea* Cyclophosphamide was not significantly associated (no effect measure reported); *Univariate odds ratio (95% CI) for amenorrhea* Cumulative cyclophosphamide dose >5 g vs. <5 g: OR 7.1 (1.5-34.0)	SB: high risk AB: low risk DB: unclear CF: low risk
	Borgman-Staudt 2012	138 childhood and adolescent HSCT survivors	Median 6 yr after HSCT (range 3-12)	Cyclophosphamide: 48%; TBI: 39%	111/133 (83%) impaired fertility (amenorrhoea, hormone substitution, ↑ FSH/ or ↓ estradiol)	*Odds ratio (95% CI) for impaired fertility* Cyclophosphamide not significantly associated (no effect measure reported)	SB: unclear AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Cyclophosphamide: 40.2%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Cyclophosphamide dose per g/m^2: RR 1.3 (1.0-2.1); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr* Cumulative cyclophosphamide dose per g/m^2: RR 1.1 (1.02-1.3)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* CED <6000 mg/m^2 vs. 0: OR 0.80 (CI 0.32-2.01); CED ≥6000 mg/m^2 vs. 0: OR 3.47 (2.08-5.78); CED without procarbazine <6000 mg/m^2 vs. 0: OR 0.71 (0.28-1.83); CED without procarbazine ≥6000 mg/m^2 vs. 0: OR 1.07 (0.50-2.28)	SB: high risk AB: low risk DB: unclear CF: high risk

			(Univariate analysis) CED without procarbazine ≤2000 mg/m²: 2/200 CCS with NSPM
GRADE assessment:			
Study design:	+4	Retrospective cohort studies	
Study limitations:	-1	Limitations: Selection bias high in 4/6, unclear in 2/9; Attrition bias low in 6/6; Detection bias unclear in 6/6; Confounding low in 5/6, high in 1/6	
Consistency:	-1	Some inconsistency, 4 studies show effect of cyclophosphamide, 1 study shows no significant effect of cyclophosphamide, and 1 study shows no significant effect of cyclophosphamide equivalence dose versus none when procarbazine is excluded	
Directness:	0	Results are direct, population and outcomes broadly generalizable	
Precision:	0	No important imprecision, large study population and number of events	
Publication bias:	0	Unlikely	
Effect size:	0	No large magnitude of effect in all studies	
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses	
Plausible confounding:	0	No plausible confounding	
Quality of evidence:		⊕⊕⊕⊖ MODERATE	
Conclusion:		Increased risk of POI after cyclophosphamide vs. no cyclophosphamide in female cancer survivors diagnosed before age 25 years. (4 studies significant effect, 1 study non-significant effect; 8,150 participants; 524 events; 5 multivariable analyses)	

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; HSCT, hematopoietic stem cell transplant; SB, selection bias; yr, year.
* Overlap in included patients in studies of Laverdiere 2005 and 2009; and Chemaitily 2006 and Levine 2018.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.4 Risk POI after higher vs. lower cyclophosphamide dose (n=4 studies)	Laverdiere 2005*	32 neuroblastoma survivors	Median 7.06 yr (range 1.9-25.5) after cancer diagnosis	Cyclophosphamide: 100%; Radiotherapy to ovaries: ±82.5%	13/32 (41%) ovarian failure (not specified)	*Odds ratio (95% CI) for ovarian failure (not specified)* Cyclophosphamide ≥7.4 g vs. <7.4 g: OR 9.62 (1.4-67.2)	SB: unclear AB: low risk DB: unclear CF: low risk
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Odds ratio (95% CI) for amenorrhea* Cumulative cyclophosphamide dose >5 g vs. <5 g: OR 7.1 (1.5-34.0)	SB: high risk AB: low risk DB: unclear CF: high risk
	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Cyclophosphamide: 40.2%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Cyclophosphamide dose per g/m²: RR 1.3 (1.0-2.1); *Relative risk (95% CI) for premature nonsurgical*	SB: high risk AB: low risk DB: unclear CF: low risk

						menopause <age 40 yr Cumulative cyclophosphamide dose per g/m²: RR 1.1 (1.02-1.3)	
	Levine 2018	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* CED <6000 mg/m² vs. 0: OR 0.80 (CI 0.32-2.01); CED ≥6000 mg/m² vs. 0: OR 3.47 (2.08-5.78); CED without procarbazine <6000 mg/m² vs. 0: OR 0.71 (0.28-1.83); CED without procarbazine ≥6000 mg/m² vs. 0: OR 1.07 (0.50-2.28) (Univariate analysis) CED without procarbazine ≤2000 mg/m²: 2/200 CCS with NSPM	SB: high risk AB: low risk DB: unclear CF: high risk

GRADE assessment:		
Study design:	+4	Retrospective cohort studies
Study limitations:	-1	Limitations: Selection bias high in 3/4, unclear in 1/3; Attrition bias low in 4/4; Detection bias unclear in 4/4; Confounding low in 2/4, high in 2/4
Consistency:	-1	Some inconsistency, 3 studies show effect of higher cyclophosphamide doses and 1 study shows no significant effect of cyclophosphamide equivalence dose when procarbazine is excluded
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large study population
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect in all studies
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊖ MODERATE
Conclusion:		Increased risk of POI after increasing doses of cyclophosphamide in female cancer survivors diagnosed before age 25 years. (3 studies significant effect, 1 study non-significant effect; 4,622 participants; 198 events; 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.
* Overlap in included patients in studies of Laverdiere 2005 and 2009.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.5 Risk POI after procarbazine (n=4 studies)	Chemaitilly 2006*	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Odds ratio (95% CI) for amenorrhea age at diagnosis 0-12 yr* Procarbazine yes vs. no: OR 3.2 (1.3-7.3) *Odds ratio (95% CI) for amenorrhea age at diagnosis 13-20 yr* Procarbazine yes vs. no: OR 2.6 (1.4-4.7)	SB: high risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2013*	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Procarbazine: 7.2%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Procarbazine dose per g/m^2: RR 2.5 (1.4-5.8)	SB: high risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2015*	108 CCS	>3 yr after cancer treatment	Any alkylating agent: 100%; Procarbazine: 21.9%; Radiotherapy to ovaries: 17.6%	8/108 (7.6%) altered ovarian function (↑ FSH, ↓ AMH and amenorrhoea)	*Mean FSH* Procarbazine dose: β 0.012, p<0.001; (Each unit increase in procarbazine dose, mean FSH values increased by 0.012 IU/L)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Procarbazine dose <4000 mg/m^2 vs. 0: OR 3.07 (0.76-12.43); Procarbazine dose ≥4000 mg/m^2 vs. 0: OR 8.96 (5.02-16.00)	SB: high risk AB: low risk DB: unclear CF: low risk

Quality of evidence:

Study design:	+4	Retrospective cohort studies
Study limitations:	-1	Limitations: Selection bias high in 4/4; Attrition bias low in 4/4; Detection bias unclear in 4/4; Confounding low in 4/4
Consistency:	0	No important inconsistency, all show effect of procarbazine
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large sample size, high total number of events and narrow confidence intervals
Publication bias:	0	Unlikely

Effect size:	0	No large magnitude of effect
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊕ HIGH
Conclusion:		Increased risk of POI after procarbazine vs. no procarbazine in female cancer survivors diagnosed before age 25 years. (4 studies significant effect; 7,134 participants; 395 events; 4 multivariable analyses)

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; SB, selection bias; yr, year.

* Overlap in included patients in studies of Chemaitily 200 and Levine 2018; and Thomas-Teinturier 2013 and 2015.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.6 Risk POI after higher vs. lower procarbazine dose (n=3 studies)	Thomas-Teinturier 2013*	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Procarbazine: 7.2%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Procarbazine dose per g/m^2: RR 2.5 (1.4-5.8)	SB: high risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2015*	108 CCS	>3 yr after cancer treatment	Any alkylating agent: 100%; Procarbazine: 21.9%; Radiotherapy to ovaries: 17.6%	8/108 (7.6%) altered ovarian function (↑ FSH, ↓ AMH and amenorrhoea)	*Mean FSH* Procarbazine dose: β 0.012, p<0.001; (Each unit increase in procarbazine dose, mean FSH values increased by 0.012 IU/L)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Procarbazine: 201 (7.2%); Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Procarbazine dose <4000 mg/m^2 vs. 0: OR 3.07 (0.76-12.43); Procarbazine dose ≥4000 mg/m^2 vs. 0: OR 8.96 (5.02-16.00)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort studies					
Study limitations:	-1	Limitations: Selection bias high in 3/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 3/3					
Consistency:	0	No important inconsistency, all show effect of higher doses of procarbazine					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, large sample size, high total number of events and narrow confidence intervals					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					

Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊕ HIGH
Conclusion:		Increased risk of POI after increasing doses of procarbazine in female cancer survivors diagnosed before age 25 years. (3 studies significant effect; 3,744 participants; 180 events; 3 multivariable analyses)

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; N/A, not applicable; SB, selection bias; yr, year.

* Overlap in included patients in studies of Thomas-Teinturier 2013 and 2015.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.7 Risk POI after busulfan (n=2 studies)	Borgman-Staudt 2012	138 childhood and adolescent HSCT survivors	Median 6 yr after HSCT (range 3-12)	Any alkylating agent: 71% Busulfan: 29%; TBI: 39%	111/133 (83%) impaired fertility (amenorrhoea, hormone substitution, ↑ FSH/ or ↓ estradiol)	*Odds ratio (95% CI) for impaired fertility* Busulfan yes vs. no: OR 47.4 (5.4-418.1)	SB: unclear AB: low risk DB: unclear CF: low risk
	Bresters 2014	109 childhood HSCT survivors	Median 7.2 yr after HSCT (>2 yr after HSCT)	Any alkylating agent: 100%; Busulfan: 31.2%; TBI: 53.2%	61/109 (56%) ovarian insufficiency (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development after age 12 yr or in post-pubertal amenorrhea)	*Relative risk (95% CI) for ovarian insufficiency* Chemotherapy with vs. without busulfan: RR 2.98 (0.99-9.03), p=0.05	SB: low risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort study					
Study limitations:	-1	Some limitations: Selection bias low in 1/1, unclear in 1/1; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2					
Consistency:	0	No important inconsistency, 1 study significant effect of busulfan, 1 study borderline significant effect of busulfan					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision, small sample size and broad confidence interval					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Unclear if dose-response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊖⊖ LOW					
Conclusion:		Increased risk of POI after busulfan vs. no busulfan in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 1 study non-significant effect; 247 participants; 172 events; 2 multivariable analysis)					

Abbreviations: AB, attrition bias; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplantation; LH, luteinizing hormone; N/A, not applicable; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
1.8 Risk POI after melphalan (n=1 study)	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Any alkylating agent: 47.7%; Melphalan: 1.3%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Melphalan yes vs. no: RR 15.2 (3.2-52.7); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr* Melphalan yes vs. no: RR 32.0 (2.0-530.0)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Retrospective cohort study
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Some imprecision, only 1 study included with a large sample size; although broad confidence intervals it does not cross the clinical decision threshold
Publication bias:	0	Unlikely
Effect size:	0	Although this study found a large magnitude of effect (lower bound 95% CI >2), there is only one study included so we do not know for sure if the effect size is truly large
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊖⊖⊖ VERY LOW
Conclusion:		Increased risk of POI after melphalan vs. no melphalan in female cancer survivors diagnosed before age 25 years. (1 study significant effect; 706 participants; 62 events; 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; N/A, not applicable; SB, selection bias; yr, year.

1.9 What is the risk of POI in female cancer patients diagnosed before age 25 years who will be treated with chlorambucil, mechloretamine, ifosfamide, thiotepa, carmustine (BCNU), lomustine (CCNU)?

No studies identified investigating the risk of POI in childhood cancer survivors treated chlorambucil, mechloretamine, ifosfamide, thiotepa, carmustine (BCNU), lomustine (CCNU)

2. What is the risk of POI in female cancer patients diagnosed before age 25 years who will be treated with antimetabolites (cytarabine, fludarabine, methotrexate)?

No studies identified investigating the risk of POI in childhood cancer survivors treated with antimetabolites.

3. What is the risk of POI in female cancer patients diagnosed before age 25 years who will be treated with platinum compounds (cisplatin, carboplatin)?
No studies identified investigating the risk of POI in childhood cancer survivors treated with platinum compounds.

4. What is the influence of age at treatment on the risk of POI in female cancer patients diagnosed before age 25 years?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
4.1 Risk POI by age at cancer diagnosis (n=11 studies)	Byrne 1992	1,048 CCS vs. 1,596 siblings	>19 yr after cancer diagnosis	Alkylating agents: at least 11.1%; Radiotherapy to ovaries: 7.5%	123/954 (12.9%) amenorrhoea after study entry	*Relative risk (95% CI) for amenorrhea* Age 0-12 at diagnosis (and aged 21-30 at follow-up) vs. controls: RR 0.62, p>0.05; Age 13-19 at diagnosis (and aged 21-30 at follow-up): RR 2.32 (1.63-3.291)	SB: unclear AB: low risk DB: unclear CF: high risk
	Chemaitilly 2006*	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Univariate odds ratio (95% CI) for amenorrhea* Age ≥12 yr at diagnosis vs. <12 yr: OR 1.8 (1.4-2.4); Significant interactions between age at diagnosis and high doses of radiotherapy to the ovary (p=0.03 for dose ≥2000cGy) and between age at diagnosis and treatment with cyclophosphamide (p=0.0006), with this drug being a significant risk factor only for the older age group in multivariable analysis	SB: high risk AB: low risk DB: unclear CF: low risk
	Sklar 2006*	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Risk for (non-surgical) amenorrhea* Age at diagnosis was not significantly associated (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: low risk
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Risk for amenorrhea* Age at diagnosis was not significantly associated (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: low risk
	Jadoul 2011	35 childhood HSCT survivors	Mean 15.5 (range 3.3-33.7) yr after HSCT	Alkylating agents: 100%; Radiotherapy to ovaries: 51.4%	21/35 (60.0%) ovarian failure 10 yr after HSCT	*Risk for ovarian failure* Independent protective effect of young age at HSCT (p=0.004) (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: unclear
	Borgman-Staudt 2012	138 childhood and adolescent	Median 6 yr after HSCT (range 3-12)	Cyclophosphamide: 48%;	111/133 (83%) impaired fertility	*Odds ratio (95% CI) for impaired fertility*	SB: unclear AB: low risk

		HSCT survivors		TBI: 39%	(amenorrhoea, hormone substitution, ↑ FSH/ or ↓ estradiol)	Pubertal patients vs pre-pubertal patients: OR 4.7 (1.5-14.9)	DB: unclear CF: low risk
	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Alkylating agents: 47.7%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Alkylating agents before pubertal period vs. none: RR 2.8 (1.2-6.5); Alkylating agents during pubertal period vs. none: RR 14.8 (4.2-52.8); Alkylating agents after menses vs. none: RR 7.6 (3.0-19.1); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr* Age at diagnosis per yr: RR 1.3 (1.04-1.6)	SB: high risk AB: low risk DB: unclear CF: low risk
	Bresters 2014	109 childhood HSCT survivors	Median 7.2 yr afer HSCT (>2 years after HSCT)	Alkylating agents: 100%; Radiotherapy to ovaries: 53.2%	61/109 (56%) ovarian insufficiency (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development after age 12 yr or in post-pubertal amenorrhea)	*Cumulative incidence ovarian insufficiency by age at HSCT* <5 yr: 35% (n=11/31); 5-10 yr: 77% (n=27/35); 15-20 yr: 79% (n=11/14); p=0.001 *Relative risk (95% CI) for ovarian insufficiency* Pubertal patients vs pre-pubertal patients: RR 4.42 (1.90-10.27) Post-pubertal vs pre-pubertal patients: RR 22.08 (9.46-51.54)	SB: low risk AB: low risk DB: unclear CF: high risk
	Vatanen 2014	92 childhood allogeneic HSCT survivors	Mean 13 (range 6-27) yr after HSCT	Alkylating agents: 100%; Radiotherapy to ovaries: 77.2%	54/92 (58.7%) no ovarian function (↑ FSH, amenorrhoea, failure to accomplish pubertal maturation)	*Odds ratio (95% CI) for no spontaneous menses* Age at HSCT: OR 1.1 (0.99-1.30)	SB: low risk AB: low risk DB: unclear CF: low risk
	Chemaitilly 2017	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Age at diagnosis: HR 0.97 (0.92-1.02)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%;	110/2930 (3.8%) nonsurgical	*Odds ratio (95% CI) for nonsurgical premature*	SB: high risk AB: low risk

			Radiotherapy to ovaries: 55.4%	premature menopause <age 40 yr	*menopause* Age at diagnosis 10-14 yr vs. 0-9 yr: 1.14 (0.63-2.06); Age at diagnosis 15-20 yr vs. 0-9 yr: 1.98 (1.16-3.38) (Univariate analysis)	DB: unclear CF: high risk
GRADE assessment:						
Study design:	+4	Retrospective cohort studies				
Study limitations:	-1	Limitations: Selection bias low in 2/11, high in 7/11, unclear in 2/11; Attrition bias low in 11/11; Detection bias unclear in 11/11; Confounding low in 7/11, high in 3/11, unclear in 1/11				
Consistency:	-1	Some inconsistency, 7 studies show significant effect of older age at cancer treatment, 4 studies show non-significant effects in different directions				
Directness:	0	Results are direct, population and outcomes broadly generalizable				
Precision:	0	No important imprecision, large sample size, high total number of events and narrow confidence intervals				
Publication bias:	0	Unlikely				
Effect size:	0	No large magnitude of effect				
Dose-response:	0	Although it seems that older ages are associated with an increased risk as compared to younger ages, we are not 100% confident				
Plausible confounding:	0	No plausible confounding				
Quality of evidence:		⊕⊕⊖⊖ LOW				
Conclusion:		Increased risk of POI after older age at cancer treatment vs. younger age in female cancer survivors diagnosed before age 25 years. (7 studies significant effect, 4 studies non-significant effect; 13,142 participants; 996 events; 7 multivariable analyses)				

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

* Overlap in included patients in studies of Laverdiere 2009, Chemaitily 2006, Sklar 2006 and Levine 2018.

5. What is the risk of POI in female cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the ovaries?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
5.1 Risk POI after radiotherapy to volumes exposing the ovaries (n=17 studies)	Byrne 1992	1,048 CCS vs. 1,596 siblings	>19 yr after cancer diagnosis	Alkylating agents: at least 11.1%; Radiotherapy to ovaries: 7.5%	123/954 (12.9%) amenorrhoea after study entry	*Relative risk (95% CI) for amenorrhea* Radiotherapy below diaphragm yes vs. no (controls): Women aged 21-25 yr: RR 3.66 (1.34-9.99); Women aged 26-30 yr: RR 2.41 (p<0.05); Women aged 31-40 yr: RR 0.90 (p>0.05); Women aged >40 yr: RR 1.22 (p>0.05)	SB: unclear AB: low risk DB: unclear CF: high risk
	Chiarelli 1999	719 CCS vs. 162 CCS with non-sterilizing surgery	5-30 yr after diagnosis	Alkylating agents: at least 20.1%; Radiotherapy to ovaries: 21.4%	63/719 (8.8%) amenorrhoea after treatment	*Risk ratio (95% CI) for amenorrhea* Abdominal-pelvic radiotherapy vs. non-sterilizing surgery: RR 1.62 (95% CI 0.80-3.28); <2000 cGy: RR 1.02 (0.29- 3.59) 2000-3499 cGy: RR 1.36 (0.57-3.25) ≥3500 cGy: RR 3.27 (1.57-6.81)	SB: high risk AB: unclear DB: unclear CF: low risk
	Wallace 2003*	27 childhood leukaemia and intra-abdominal tumour survivors	NM	Alkylating agents: at least 40.7%; Radiotherapy to ovaries: 100%	24/27 (88.9%) ovarian failure (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development or in post-pubertal amenorrhea)	A dose of 1.99 Gy to the abdomen is required to destroy 50% of the oocytes based on the Faddy-Gosden mathematical model	SB: unclear AB: low risk DB: unclear CF: high risk
	Wallace 2005*	27 childhood leukaemia and intra-abdominal tumour survivors	NM	Alkylating agents: at least 40.7%; Radiotherapy to ovaries: 100%	24/27 (88.9%) ovarian failure (↑ FSH/LH, ↓ estradiol; In pre-pubertal females	A dose of 20.3 Gy to the ovaries at birth is associated with POI in 97.5% of the patients; A dose of 18.4 Gy to the	SB: unclear AB: low risk DB: unclear CF: high risk

					absence of spontaneous pubertal development or in post-pubertal amenorrhea)	ovaries at 10 years of age is associated with POI in 97.5% of the patients; A dose of 16.5 Gy to the ovaries at 20 years of age is associated with POI in 97.5% of the patients, based on the Faddy-Gosden mathematical model	
	Chemaitilly 2006*	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Odds ratio (95% CI) for amenorrhea age at diagnosis 0-12 yr* Radiotherapy to ovaries 1-99 vs. 0 cGy: OR 3.7 (1.6-10.2) Radiotherapy to ovaries 100-999 vs. 0 cGy: OR 9.0 (3.4-26.5) Radiotherapy to ovaries 1000-1999 vs. 0 cGy: OR 55.3 (22.3-157.8) Radiotherapy to ovaries ≥2000 vs. 0 cGy: OR 950.1 (352.9-3043.2) *Odds ratio (95% CI) for amenorrhea age at diagnosis 13-20 yr* Radiotherapy to ovaries 1-99 vs. 0 cGy: OR 2.9 (1.2-8.3) Radiotherapy to ovaries 100-999 vs. 0 cGy: OR 17.2 (6.8-49.5) Radiotherapy to ovaries 1000-1999 vs. 0 cGy: OR 90.9 (29.1-323.5) Radiotherapy to ovaries ≥2000 vs. 0 cGy: OR 171.2 (55.8-609.8)	SB: high risk AB: low risk DB: unclear CF: low risk
	Sklar 2006*	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Risk ratio (95% CI) for (non-surgical) amenorrhea* Radiotherapy to ovaries 1-99 vs. 0 cGy: RR 4.30 (1.20-15.47); Radiotherapy to ovaries 100-999 vs. 0 cGy: RR 5.70 (1.12-28.99);	SB: high risk AB: low risk DB: unclear CF: low risk

						Radiotherapy to ovaries ≥1000 vs. 0 cGy: RR 109.59 (28.15-426.70)	
	Laverdiere 2009*	954 neuroblastoma survivors	>5 yr after cancer diagnosis	Alkylating agents: NM; Radiotherapy to ovaries: NM	13/204 (6.4%) amenorrhea after treatment	*Risk for amenorrhea* Radiotherapy to ovaries was significantly associated p<0.005 (no effect measure reported); *Univariate odds ratio (95% CI) for amenorrhea* Radiotherapy to ovaries: OR 8.4 (1.1-67.7)	SB: high risk AB: low risk DB: unclear CF: low risk
	Jadoul 2011	35 childhood HSCT survivors	Mean 15.5 (range 3.3-33.7) yr after HSCT	Alkylating agents: 100%; Radiotherapy to ovaries: 51.4%	21/35 (60.0%) ovarian failure 10 yr after HSCT	*Risk for ovarian failure* Independent negative effect of TBI (p=0.014) (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: unclear
	Gracia 2012	71 CCS vs. 67 postmenarchal controls	>1 yr after cancer treatment	Alkylating agents: 88.7%; Radiotherapy to ovaries: 18.3%	NM 49 (69.0%) regular cycles	*Geometric mean FSH* Pelvic radiation vs. controls: 28.4 vs. 9.4 mIU/mL, p<0.001	SB: unclear AB: low risk DB: unclear CF: low risk
	Borgman-Staudt 2012	138 childhood and adolescent HSCT survivors	Median 6 yr after HSCT (range 3-12)	Cyclophosphamide: 48%; TBI: 39%	111/133 (83%) impaired fertility (amenorrhoea, hormone substitution, ↑ FSH/ or ↓ estradiol)	*Odds ratio (95% CI) for impaired fertility* TBI vs. no TBI: OR 4.9 (1.2-19.9)	SB: unclear AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2013*	706 CCS	>5 yr after cancer diagnosis	Alkylating agents: 47.7%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Minimal radiation dose to ovaries per Gy: RR 1.1 (1.0-1.6); Minimal radiation dose to ovaries 0.01-<1 vs. <0.01 Gy: RR 1.3 (0.6-2.9); Minimal radiation dose to ovaries 1-<10 vs. <0.01 Gy: RR 2.3 (1.0-5.1); Minimal radiation dose to ovaries ≥10 Gy vs. <0.01 Gy: RR 3.8 (1.2-11.6); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr*	SB: high risk AB: low risk DB: unclear CF: low risk

						Radiation dose to ovaries per Gy: RR 1.1 (1.0-1.2)	
	Bresters 2014	109 childhood HSCT survivors	Median 7.2 yr after HSCT (>2 years after HSCT)	Alkylating agents: 100%; Radiotherapy to ovaries: 53.2%	61/109 (56%) ovarian insufficiency (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development after age 12 yr or in post-pubertal amenorrhea)	*Relative risk (95% CI) for ovarian insufficiency* TBI/TAI vs. alkylating agents only: RR 0.77 (0.44-1.35)	SB: low risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2015*	108 CCS vs. 20 healthy menstruating females	>3 yr after cancer treatment	Alkylating agents: 100%; Radiotherapy to ovaries: 17.6%	8/108 (7.6%) altered ovarian function (↑ FSH, ↓ AMH and amenorrhea)	*Mean FSH* Significant higher FSH levels in CCS treated with alkylating agents + subdiaphragmatic radiotherapy vs. alkylating agents alone, p=0.009; Significant higher FSH levels in CCS treated with alkylating agents + subdiaphragmatic radiotherapy vs. controls, p=0.0009 (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: high risk
	Vatanen 2014	92 childhood allogeneic HSCT survivors	Mean 13 (range 6-27) yr after HSCT	Alkylating agents: 100%; Radiotherapy to ovaries: 77.2%	54/92 (58.7%) no ovarian function (↑ FSH, amenorrhoea, failure to accomplish pubertal maturation)	*Odds ratio (95% CI) for no spontaneous menses* TBI yes vs. no: OR 5.2 (1.6-16.5)	SB: low risk AB: low risk DB: unclear CF: low risk
	Chemaitilly 2017*	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhoea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Ovarian radiation dose >999 vs. 0 cGy: HR 13.85 (6.50-29.51); Ovarian radiation dose ≥1000 vs. 0 cGy: HR 132.34 (62.88-278.53); Ovarian radiation only vs. no alkylating agents nor ovarian radiotherapy: HR 71.7 (16.50-	SB: high risk AB: low risk DB: unclear CF: low risk

						311.58); Alkylating agents and ovarian radiation vs. no alkylating agents nor ovarian radiotherapy: HR 95.56 (23.30-391.93)	
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Minimum ovarian radiation dose >0-500 cGy vs. 0: OR 2.73 (95% CI 1.33-5.61); Minimum ovarian radiation dose >500 cGy vs. 0: OR 8.02 (95% CI 2.81-22.85)	SB: high risk AB: low risk DB: unclear CF: low risk
	Fernandez Pineda 2018*	90 childhood Hodgkin lymphoma survivors	>10 yr after cancer diagnosis	Alkylating agents: 97%; Radiotherapy to ovaries: 100%	Events NM (premature ovarian insufficiency defined as absence of menses 5 years post cancer diagnosis or loss of spontaneous menses prior to 40 years of age with laboratory or historic evidence of primary (ovarian) origin)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Pelvic radiation dose ≤1,500 vs. >1,500 cGy: HR 25.2 (CI 3.1-207.3)	SB: low risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Retrospective cohort studies
Study limitations:	-1	Limitations: Selection bias low in 3/17, high in 9/17, unclear in 5/17; Attrition bias low in 16/17, unclear in 1/17; Detection bias unclear in 17/17; Confounding low in 12/17, high in 4/17, unclear in 1/17
Consistency:	0	No important inconsistency, all show effect of radiotherapy to the ovaries (1 study non-significant effect)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large sample size and high total number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect in all studies
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊕ HIGH
Conclusion:		Increased risk of POI after radiotherapy to volumes exposing the ovaries vs. no radiotherapy in female cancer survivors diagnosed before age 25 years. (17 studies significant effect, 1 study non-significant effect; 14,184 participants; 1,115 events; 12 multivariable analyses)

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

* Overlap in included patients in studies of Wallace 2003 and 2005; and Laverdiere 2009, Chemaitily 2006, Sklar 2006 and Levine 2018; Chemaitily 2017 and Fernandez-Pineda 2018; and Thomas-Teinturier 2013 and 2015.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
5.2 Risk POI after higher vs. lower doses radiotherapy to volumes exposing the ovaries (n=9 studies)	Chiarelli 1999	719 CCS vs. 162 CCS with non-sterilizing surgery	5-30 yr after diagnosis	Alkylating agents: at least 20.1%; Radiotherapy to ovaries: 21.4%	63/719 (8.8%) amenorrhoea after treatment	*Risk ratio (95% CI) for amenorrhea* Abdominal-pelvic radiotherapy vs. non-sterilizing surgery: <2000 cGy: RR 1.02 (0.29- 3.59) 2000-3499 cGy: RR 1.36 (0.57-3.25) ≥3500 cGy: RR 3.27 (1.57-6.81)	SB: high risk AB: unclear DB: unclear CF: low risk
	Wallace 2003*	27 childhood leukaemia and intra-abdominal tumour survivors	NM	Alkylating agents: at least 40.7%; Radiotherapy to ovaries: 100%	24/27 (88.9%) ovarian failure (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development or in post-pubertal amenorrhea)	A dose of 1.99 Gy to the abdomen is required to destroy 50% of the oocytes based on the Faddy-Gosden mathematical model	SB: unclear AB: low risk DB: unclear CF: high risk
	Wallace 2005*	27 childhood leukaemia and intra-abdominal tumour survivors	NM	Alkylating agents: at least 40.7%; Radiotherapy to ovaries: 100%	24/27 (88.9%) ovarian failure (↑ FSH/LH, ↓ estradiol; In pre-pubertal females absence of spontaneous pubertal development or in post-pubertal amenorrhea)	A dose of 20.3 Gy to the ovaries at birth is associated with POI in 97.5% of the patients; A dose of 18.4 Gy to the ovaries at 10 years of age is associated with POI in 97.5% of the patients; A dose of 16.5 Gy to the ovaries at 20 years of age is associated with POI in 97.5% of the patients, based on the Faddy-Gosden mathematical model	SB: unclear AB: low risk DB: unclear CF: high risk
	Chemaitilly 2006*	3,390 CCS	>5 yr after cancer diagnosis	Alkylating agents: 49.7%; Radiotherapy to ovaries: 24.5%	215/3390 (6.3%) amenorrhea within 5 yr after their cancer diagnosis	*Odds ratio (95% CI) for amenorrhea age at diagnosis 0-12 yr* Radiotherapy to ovaries 1-99 vs. 0 cGy: OR 3.7 (1.6-10.2) Radiotherapy to ovaries 100-999 vs. 0 cGy: OR 9.0 (3.4-26.5)	SB: high risk AB: low risk DB: unclear CF: low risk

						Radiotherapy to ovaries 1000-1999 vs. 0 cGy: OR 55.3 (22.3-157.8) Radiotherapy to ovaries ≥2000 vs. 0 cGy: OR 950.1 (352.9-3043.2) *Odds ratio (95% CI) for amenorrhea age at diagnosis 13-20 yr* Radiotherapy to ovaries 1-99 vs. 0 cGy: OR 2.9 (1.2-8.3) Radiotherapy to ovaries 100-999 vs. 0 cGy: OR 17.2 (6.8-49.5) Radiotherapy to ovaries 1000-1999 vs. 0 cGy: OR 90.9 (29.1-323.5) Radiotherapy to ovaries ≥2000 vs. 0 cGy: OR 171.2 (55.8-609.8)	
	Sklar 2006*	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Risk ratio (95% CI) for (non-surgical) amenorrhea* Radiotherapy to ovaries 1-99 vs. 0 cGy: RR 4.30 (1.20-15.47); Radiotherapy to ovaries 100-999 vs. 0 cGy: RR 5.70 (1.12-28.99); Radiotherapy to ovaries ≥1000 vs. 0 cGy: RR 109.59 (28.15-426.70)	SB: high risk AB: low risk DB: unclear CF: low risk
	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Alkylating agents: 47.7%; Radiotherapy to ovaries: 56.7%	62/706 (8.9%) nonsurgical menopause; 15/706 (2.1%) nonsurgical premature menopause <age 40 yr	*Relative risk (95% CI) for nonsurgical menopause* Minimal radiation dose to ovaries per Gy: RR 1.1 (1.0-1.6); Minimal radiation dose to ovaries 0.01-<1 vs. <0.01 Gy: RR 1.3 (0.6-2.9); Minimal radiation dose to ovaries 1-<10 vs. <0.01 Gy: RR 2.3 (1.0-5.1); Minimal radiation dose to ovaries ≥10 Gy vs. <0.01 Gy: RR 3.8 (1.2-11.6); *Relative risk (95% CI) for premature nonsurgical menopause <age 40 yr*	SB: high risk AB: low risk DB: unclear CF: low risk

					Radiation dose to ovaries per Gy: RR 1.1 (1.0-1.2)	
	Chemaitilly 2017*	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhoea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Ovarian radiation dose >999 vs. 0 cGy: HR 13.85 (6.50-29.51); Ovarian radiation dose ≥1000 vs. 0 cGy: HR 132.34 (62.88-278.53);	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Minimum ovarian radiation dose >0-500 cGy vs. 0: OR 2.73 (95% CI 1.33-5.61); Minimum ovarian radiation dose >500 cGy vs. 0: OR 8.02 (95% CI 2.81-22.85)	SB: high risk AB: low risk DB: unclear CF: low risk
	Fernandez Pineda 2018*	90 childhood Hodgkin lymphoma survivors	>10 yr after cancer diagnosis	Alkylating agents: 97%; Radiotherapy to ovaries: 100%	Events NM (premature ovarian insufficiency defined as absence of menses 5 years post cancer diagnosis or loss of spontaneous menses prior to 40 years of age with laboratory or historic evidence of primary (ovarian) origin)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Pelvic radiation dose ≤1,500 vs. >1,500 cGy: HR 25.2 (CI 3.1-207.3)	SB: low risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Retrospective cohort studies
Study limitations:	-1	Limitations: Selection bias low in high in 7/9, unclear in 2/9; Attrition bias low in 8/9, unclear in 1/9; Detection bias unclear in 9/9; Confounding low in 7/9, high in 2/9
Consistency:	0	No important inconsistency, all show that higher doses of radiotherapy to the ovaries are associated with higher risk
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, large sample size and high total number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect in all studies
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊕ HIGH
Conclusion:		Increased risk of POI after increasing doses of radiotherapy to volumes exposing the ovaries in female cancer survivors diagnosed before age 25 years. (9 studies significant effect; 11,629 participants; 724 events; 7 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.
* Overlap in included patients in studies of Wallace 2003 and 2005; and Chemaitily 2006, Sklar 2006 and Levine 2018; and Chemaitily 2017 and Fernandez-Pineda 2018.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
5.3 Risk POI after radiotherapy to volumes exposing the ovaries and alkylating agents vs. either treatment alone (n=3 studies)	Sklar 2006	2,819 CCS	>5 yr after cancer diagnosis	Alkylating agents: 48.2%; Radiotherapy to ovaries: 52.2%	126/2819 (4.4%) amenorrhea after treatment	*Cumulative incidence non-surgical premature menopause* Alkylating agents only: ± 15%; Abdominopelvic radiotherapy only: ± 5%; Alkylating agents and abdominopelvic radiotherapy: ± 30%	SB: high risk AB: low risk DB: unclear CF: high risk
	Thomas-Teinturier 2015	108 CCS vs. 20 healthy menstruating females	>3 yr after cancer treatment	Alkylating agents: 100%; Radiotherapy to ovaries: 17.6%	8/108 (7.6%) altered ovarian function (↑ FSH, ↓ AMH and amenorrhea)	*Mean FSH* Significant higher FSH levels in CCS treated with alkylating agents + subdiaphragmatic radiotherapy vs. alkylating agents alone, p=0.009; (no effect measure reported)	SB: high risk AB: low risk DB: unclear CF: high risk
	Chemaitilly 2017	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer diagnosis	Alkylating agents: 58.8%; Radiotherapy to ovaries: 17.9%	100/921 (10.9%) premature ovarian insufficiency (amenorrhoea, ↑ FSH, ↓ estradiol)	*Hazard ratio (95% CI) for premature ovarian insufficiency* Alkylating agents only vs. no alkylating agents nor ovarian radiotherapy: HR 2.98 (0.63-14.06); Ovarian radiation only vs. no alkylating agents nor ovarian radiotherapy: HR 71.7 (16.50-311.58); Alkylating agents and ovarian radiation vs. no alkylating agents nor ovarian radiotherapy: HR 95.56 (23.30-391.93)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort studies					
Study limitations:	-2	Serious limitations: Selection bias high in 3/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 1/3, high in 2/3					
Consistency:	0	No important inconsistency, all show higher risk in after alkylating agents and radiotherapy to the ovaries vs. either treatment alone					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, large sample size and high total number of events					

Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊖ MODERATE
Conclusion:		Increased risk of POI after radiotherapy to volumes exposing the ovaries and alkylating agents vs. either treatment in the same dose alone in female cancer survivors diagnosed before age 25 years. (2 studies significant effect, 1 no statistical test; 3,848 participants; 234 events; 1 multivariable analysis)

Abbreviations: AB, attrition bias; AMH, anti-Müllerian hormone; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; HSCT, hematopoietic stem cell transplant; SB, selection bias; yr, year.

6. **What is the risk of hypogonadotropic hypogonadism in female cancer patients diagnosed before age 25 years after treatment with radiotherapy to the field that includes the hypothalamic-pituitary axis?**
   - **What is the risk in younger vs older patients?**
   - **What is the risk after higher doses vs lower doses?**
   - **What is the risk after conventional vs proton therapy?**

Evidence from IGHG hypothalamic-pituitary disorders surveillance guideline; note that this is evidence for both males and females

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy Alkylating agents	Events	Effect size	Risk of bias
6.1 Risk hypogonadotropic hypogonadism in males and females after cranial radiotherapy (n=1 study)	Gan 2015	116 male and female childhood optic glioma survivors	Median 8.3 yr (range 0.04-26.8)	Cranial radiotherapy: 59.5%; Alkylating agents: NM	21/103 (20.4%) central hypogonadism (boys: testicular volume <4mL at age 14 yr or failure to progress through puberty after normal onset; girls: tanner breast stage B1 at age 13 yr or pubertal arrest or primary amenorrhea at age 16 yr	*Hazard ratio (95% CI) for central hypogonadism* Primary radiotherapy yes vs. no: HR 3.27 (1.35-7.94)	SB: low risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	0	No important limitations: Selection bias low in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-2	Important imprecision, only 1 study included and low number of events					

Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Increased risk of hypogonadotropic hypogonadism after cranial radiotherapy vs. no cranial radiotherapy in female brain tumour survivors diagnosed before age 25 years. (1 study significant effect, 116 participants, 21 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; NM, not mentioned; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Cranial radiotherapy Alkylating agents	Events	Effect size	Risk of bias
6.2 Risk hypogonadotropic hypogonadism in males and females after higher vs. lower doses of cranial radiotherapy (n=1 study)	Chemaitilly 2015	748 male and female CCS treated with cranial radiotherapy	Mean 27.3 yr (range 10.8-47.7) after cancer diagnosis	Cranial radiotherapy: 100%; Alkylating agents: NM	79/731 (10.8%) central hypogonadism (males: ↓ testosterone and ↓ LH; females: amenorrhea or ↓ estradiol and ↓ FSH)	*Odds ratio (95% CI) for central hypogonadism* Cranial radiotherapy dose 22-29.9 Gy vs. ≤21.9 Gy: OR 3.02 (1.3-7.0); Cranial radiotherapy dose ≥30 Gy vs. ≤21.9 Gy: OR 9.71 (4.2-22.3)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only 1 study included but high number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	+1	Dose response relationship as higher doses are associated with an increased risk as compared to lower doses
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊕⊖ MODERATE
Conclusion:		Increased risk of hypogonadotropic hypogonadism after increasing doses of cranial radiotherapy in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 748 participants, 79 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; ALL, acute lymphoblastic leukaemia; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; LH, luteinizing hormone; NM, not mentioned; SB, selection bias; yr, year.

7. What is the risk of POI after (partial) unilateral oophorectomy (either for purposes of fertility preservation, malignant cause or non-malignant cause)?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries Oophorectomy	Events	Effect size	Risk of bias
7.1 Risk POI after unilateral oophorectomy (n=2 studies)	Thomas-Teinturier 2013	706 CCS	>5 yr after cancer diagnosis	Alkylating agents: 47.7%; Radiotherapy to ovaries: 56.7%; Unilateral oophorectomy: 5.7%	62/706 (8.9%) nonsurgical menopause	*Relative risk (95% CI) for nonsurgical menopause* Unilateral oophorectomy yes vs. no: RR 3.7 (1.1-11.2)	SB: high risk AB: low risk DB: unclear CF: low risk
	Levine 2018	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Radiotherapy to ovaries: 55.4%; Unilateral oophorectomy: 2.1%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) nonsurgical premature menopause* Unilateral oophorectomy yes vs. no: 1.52 (0.56-4.07)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort studies					
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2					
Consistency:	-1	Some inconsistency, 1 study significant effect of unilateral oophorectomy and 1 study non-significant effect					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Unclear if dose-response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:	⊕⊕⊖⊖ LOW						
Conclusion:	Increased risk of POI after unilateral oophorectomy vs. no oophorectomy in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 1 study non-significant effect; 3,636 participants; 172 events; 2 multivariable analyses)						

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries Oophoropexy	Events	Effect size	Risk of bias
7.2 Risk POI after oophoropexy	Chemaitilly 2017	921 CCS	Median 24.0 (range 10.2-48.1) yr after cancer	Alkylating agents: 58.8%; Radiotherapy to	100/921 (10.9%) premature ovarian insufficiency	*Hazard ratio (95% CI) for premature ovarian insufficiency*	SB: high risk AB: low risk DB: unclear

(n=1 study)			diagnosis	ovaries: 17.9%; Oophoropexy: 6.3%	(amenorrhoea, ↑ FSH, ↓ estradiol)	Oophoropexy yes vs. no: HR 1.33 (0.70-2.53) (in model with separate treatment modalities); HR 0.72 (0.42-1.23) (in model with combining treatment modalities)	CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-2	Important imprecision, only 1 study included in which the confidence interval crosses the clinical decision threshold					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Unclear if dose-response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊖⊖⊖ VERY LOW					
Conclusion:		No significant effect of oophoropexy vs. no oophoropexy on the risk of POI in female cancer survivors diagnosed before age 25 years. (1 study non-significant effect; 921 participants; 100 events; 1 multivariable analysis)					

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; FSH, follicle stimulating hormone; N/A, not applicable; SB, selection bias; yr, year.

8. What is the risk of POI in female cancer patients diagnosed before age 25 years after stem cell transplant?

- **What is the risk after autologolous vs allogeneic transplant?**
- **What is the risk after reduced conditioning vs myeloablative?**

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy to ovaries	Events	Effect size	Risk of bias
8.1 Risk POI after HSCT (n=1 study)	Levine 2018*	2,930 CCS	>5 yr after cancer diagnosis	Alkylating agents: 46.5%; Radiotherapy to ovaries: 55.4%	110/2930 (3.8%) nonsurgical premature menopause <age 40 yr	*Odds ratio (95% CI) for nonsurgical premature menopause* Stem cell transplant yes vs. no: OR 6.35 (1.19-33.93)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Retrospective cohort studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	N/A (1 study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-2	Some imprecision, only 1 study included and broad confidence intervals					
Publication bias:	0	Unlikely					

Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊖⊖⊖ VERY LOW
Conclusion:		Increased risk of POI after stem cell transplant vs. no stem cell transplant independent of alkylating agents and/or radiotherapy to volumes exposing the ovaries in female cancer survivors diagnosed before age 25 years. (1 study; 2,930 participants; 110 events; 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; N/A, not applicable; SB, selection bias; yr, year.

9. What is the risk of POI in female cancer patients diagnosed before age 25 years after treatment with:

- **Anthracyclines**
- **Novel therapies: monoclonal antibodies, tyrosine kinases inhibitors**
- **High-dose etoposide**

No studies identified investigating the risk of POI in childhood cancer survivors treated with anthracyclines, novel therapies, or high-dose etoposide.

10. What is the risk of POI in female cancer patients diagnosed before age 25 years with a genetic predisposition after treatment with:

- **Radiotherapy to volumes exposing the ovaries and/or (cranio)spinal radiotherapy**
- **Chemotherapy**

No studies identified investigating the risk of POI in childhood cancer survivors with a genetic predisposition.

11. What is the risk of POI in female cancer patients diagnosed before age 25 years with 1 vs. 2 ovaries in the radiotherapy field?

- **What is the risk in younger vs older patients?**

No studies identified investigating the risk of POI in childhood cancer survivors with 1 vs. 2 ovaries in the radiotherapy field.

12. What is the likelihood of a pregnancy/live birth among female cancer patients diagnosed before age 25 years who will be treated with alkylating agents?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.1 Likelihood pregnancy and live birth after cyclophosphamide and higher vs. lower doses	Chow 2016*	5298 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 53%; Radiotherapy to pelvis, brain or total body: 0%	2455/5298 (46%) reported at least 1 pregnancy; 2028/5298 (38%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Cyclophosphamide lower tertile dose (<3625 mg/m^2) vs. 0: HR 0.92 (0.82-1.04); Cyclophosphamide middle tertile	SB: high risk AB: low risk DB: unclear CF: low risk

(n=3 studies)		dose (3625-7411 mg/m²) vs. 0: HR 1.04 (0.91-1.19); Cyclophosphamide upper tertile dose (>7411 mg/m²) vs. 0: HR 0.99 (0.87-1.12); Cyclophosphamide equivalent lower tertile dose (<4897 mg/m²) vs. 0: HR 0.97 (0.86-1.08); Cyclophosphamide equivalent middle tertile dose (4897-9638 mg/m²) vs. 0: HR 0.98 (0.87-1.11); Cyclophosphamide equivalent upper tertile dose (>9638 mg/m²) vs. 0: HR 0.90 (0.79-1.01); Cyclophosphamide equivalent linear dose per 5000 mg/m²: HR 0.97 (0.94-1.00) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Cyclophosphamide lower tertile dose (<3625 mg/m²) vs. 0: HR 0.93 (0.81-1.06); Cyclophosphamide middle tertile dose (3625-7411 mg/m²) vs. 0: HR 1.06 (0.92-1.22); Cyclophosphamide upper tertile dose (>7411 mg/m²) vs. 0: HR 0.99 (0.87-1.13); Cyclophosphamide equivalent lower tertile dose (<3625 mg/m²) vs. 0: HR 0.95 (0.84-1.08); Cyclophosphamide equivalent middle tertile dose (3625-7411 mg/m²) vs. 0: HR 1.01 (0.89-1.16); Cyclophosphamide equivalent upper tertile dose (>7411 mg/m²)

						vs. 0: HR 0.91 (0.80-1.03) Cyclophosphamide equivalent linear dose per 5000 mg/m²: HR 0.97 (0.94-1.00)	
	Green 2009*	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Cyclophosphamide yes vs. no: RR 0.80 (0.68-0.93); Alkylating agent dose score 1 vs. 0: RR 0.90 (0.69-1.18); Alkylating agent dose score 2 vs. 0: RR 0.91 (0.72-1.16); Alkylating agent dose score 3 vs. 0: RR 0.72 (0.58-0.90); Alkylating agent dose score 4 vs. 0: RR 0.65 (0.45-0.96); Alkylating agent dose score 5 vs. 0: RR 0.82 (0.55-1.24); Alkylating agent dose score 6-11 vs. 0: RR 0.76 (0.49-1.19)	SB: high risk AB: low risk DB: unclear CF: low risk
	Bramswig 2015	467 Hodgkin lymphoma survivors	Median 20.4 (range 5.1-34.5) yr	Alkylating agents: 84.4%; Radiotherapy to ovaries/uterus: 7.9%; Cranial radiotherapy: 0%	228/467 (49%) females with 406 children (median 1.78 children, range 1-7)	*Hazard ratio (95% CI) for likelihood of parenthood* Alkylating agent dose score 1 vs. 0: HR 0.92 (0.62-1.37); Alkylating agent dose score 2 vs. 0: HR 0.95 (0.70-1.29); Alkylating agent dose score 3 vs. 0: HR 1.00 (0.86-1.01); Alkylating agent dose score 5 vs. 0: HR 0.93 (0.86-1.01)	SB: low AB: low DB: unclear CF: low
GRADE assessment:							

Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 2/3, low in 1/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 3/3
Consistency:	-1	Some inconsistency, one study showed significant decreased likelihood of pregnancy after (higher doses of) alkylating agents, the other two studies showed non-significant effects.
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after (increasing doses of) cyclophosphamide in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 2 studies non-significant effects, 10,914 participants, 2,683 events, 3 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

* Overlap in included patients in studies of Green 2009 and Chow 2016.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.2 Likelihood pregnancy and live birth after ifosfamide (n=1 study)	Chow 2016	5298 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 53%; Radiotherapy to pelvis, brain or total body: 0%	2455/5298 (46%) reported at least 1 pregnancy; 2028/5298 (38%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Ifosfamide lower tertile dose (<26853 mg/m²) vs. 0: HR 0.92 (0.64-1.30) Ifosfamide middle tertile dose (26853-52999 mg/m²) vs. 0: HR 0.82 (0.58-1.18) Ifosfamide upper tertile dose (>52999 mg/m²) vs. 0: HR 1.05 (0.74-1.48) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Ifosfamide lower tertile dose (<26853 mg/m²) vs. 0: HR 0.86 (0.58-1.27) Ifosfamide middle tertile dose (26853-52999 mg/m²) vs. 0: HR 0.84 (0.57-1.24) Ifosfamide upper tertile dose (>52999 mg/m²) vs. 0: HR 1.03	SB: high risk AB: low risk DB: unclear CF: low risk

		(0.70-1.50)
GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 2/2; Confounding low in 1/1
Consistency:	0	Not applicable (one study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only one study included but with high number of participants and events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		No significant effect of ifosfamide (dose) on the likelihood of pregnancy and live birth in female cancer survivors diagnosed before age 25 years. (1study no significant effect, 5,298 participants, 2,455 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.3 Likelihood pregnancy and live birth after busulfan (n=1 study)	Chow 2016	5298 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 53%; Radiotherapy to pelvis, brain or total body: 0%	2455/5298 (46%) reported at least 1 pregnancy; 2028/5298 (38%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Busulfan lower dose (<450 mg/m²) vs. 0: HR 0.22 (0.06-0.79) Busulfan upper dose (≥450 mg/m²) vs. 0: HR: 0.14 (0.03-0.55) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Busulfan lower dose (<450 mg/m²) vs. 0: HR 0.20 (0.05-0.82) Busulfan upper dose (≥450 mg/m²) vs. 0: HR: 0.18 (0.04-0.71)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					
Consistency:	0	Not applicable (one study)					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision, only one study included but with high number of participants and events					
Publication bias:	0	Unlikely					

Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy and live birth after (increasing doses of) busulfan in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 5,298 participants, 2,455 events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.4 Likelihood pregnancy and live birth after lomustine (n=2 studies)	Chow 2016*	5298 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 53%; Radiotherapy to pelvis, brain or total body: 0%	2455/5298 (46%) reported at least 1 pregnancy; 2028/5298 (38%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Lomustine lower dose (<411 mg/m²) vs. 0: HR 0.87 (0.46-1.65) Lomustine upper dose (≥411 mg/m²) vs. 0: HR: 0.41 (0.17-0.98) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Lomustine lower dose (<411 mg/m²) vs. 0: HR 1.12 (0.59-2.13) Lomustine upper dose (≥411 mg/m²) vs. 0: HR: 0.60 (0.27-1.34)	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2009*	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Lomustine yes vs. no: RR 0.44 (0.24-0.80)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency, however, both studies are from the same cohort
Directness:	0	Results are direct, population and outcomes broadly generalizable

Precision:	-1	Some imprecision, both studies are from the same cohort, but there is a high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy after (increasing doses) of lomustine in female cancer survivors diagnosed before age 25 years. (2 studies from 1 cohort significant effect, 10,447 participants, 2,455 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.
* Overlap in included patients in studies of Green 2009 and Chow 2016.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.5 Likelihood pregnancy and live birth after procarbazine (n=3 studies)	Chow 2016*	5298 CCS	Median 8 yr (inter quartile range 4-12)	Alkylating agents: 53%; Radiotherapy to pelvis, brain or total body: 0%	2455/5298 (46%) reported at least 1 pregnancy; 2028/5298 (38%) reported at least 1 live birth	*Hazard ratio (95% CI) for likelihood of reporting first pregnancy* Procarbazine lower tertile dose (<3352 mg/m²) vs. 0: HR 0.99 (0.74-1.32); Procarbazine middle tertile dose (3352-5059 g/m²) vs. 0: HR 0.97 (0.74-1.26); Procarbazine upper tertile dose (>5059 mg/m²) vs. 0: HR 0.93 (0.70-1.22) *Hazard ratio (95% CI) for likelihood of reporting first live birth* Procarbazine lower tertile dose (<3352 mg/m²) vs. 0: HR 0.87 (0.64-1.20); Procarbazine middle tertile dose (3352-5059 g/m²) vs. 0: HR 1.03 (0.97-1.35); Procarbazine upper tertile dose (>5059 mg/m²) vs. 0: HR 0.78 (0.58-1.05)	SB: high risk AB: low risk DB: unclear CF: low risk
	Green 2009*	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy*	SB: high risk AB: low risk DB: unclear

				ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%		Procarbazine yes vs. no: RR 0.94 (0.68-1.31)	CF: low risk
	Bramswig 2015	467 Hodgkin lymphoma survivors	Median 20.4 (range 5.1-34.5) yr	Alkylating agents: 84.4%; Radiotherapy to ovaries/uterus: 7.9%; Cranial radiotherapy: 0%	228/467 (49%) females with 406 children (median 1.78 children, range 1-7)	*Hazard ratio (95% CI) for likelihood of parenthood* Procarbazine 2 cycles vs. 0: HR 0.96 (0.80-1.16); Procarbazine 4 cycles vs. 0: HR 1.01 (0.91-1.12); Procarbazine 6-8 cycles vs. 0: HR 0.94 (0.88-1.01)	SB: low AB: low DB: unclear CF: low
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 2/3, low in 1/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 3/3					
Consistency:	0	No important inconsistency, all three studies showed non-significant effects					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	0	No important imprecision, high total number of included patients and events and narrow confidence intervals					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	Unclear if dose-response relationship					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:		⊕⊕⊕⊖ MODERATE					
Conclusion:		No significant effect of procarbazine (dose) on the likelihood of pregnancy and live birth in female cancer survivors diagnosed before age 25 years. (3 studies no significant effect, 10,914 participants, 2,683 events, 3 multivariable analyses)					

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.
* Overlap in included patients in studies of Green 2009 and Chow 2016.

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
12.5 Likelihood pregnancy and live birth after mechlorethamine (n=1 study)	Green 2009	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Mechlorethamine yes vs. no: RR 0.82 (0.57-1.19)	SB: high risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1					

Consistency:	0	Not applicable (one study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only one study, but a high total number of included patients and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		No significant effect of mechlorethamine on the likelihood of pregnancy in female cancer survivors diagnosed before age 25 years. (1 study no significant effect, 5,149 participants, unclear number of events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

13. What is the likelihood of a pregnancy/live birth among female cancer patients diagnosed before age 25 years who will be treated with radiotherapy to volumes exposing the ovaries?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
13.1 Likelihood pregnancy and live birth after radiotherapy to volumes exposing the ovaries (n=3 studies)	Green 2009	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Radiotherapy to ovaries/uterus 2.5-5.0 Gy vs. ≤2.5 Gy: RR 0.80 (0.57-1.11); Radiotherapy to ovaries/uterus 5.0-10.0 Gy vs. ≤2.5 Gy: RR 0.56 (0.37-0.85); Radiotherapy to ovaries/uterus >10.0 Gy vs. ≤2.5 Gy: RR 0.18 (0.13-0.26)	SB: high risk AB: low risk DB: unclear CF: low risk
	Bramswig 2015	467 Hodgkin lymphoma survivors	Median 20.4 (range 5.1-34.5) yr	Alkylating agents: 84.4%; Radiotherapy to ovaries/uterus: 7.9%; Cranial radiotherapy: 0%	228/467 (49%) females with 406 children (median 1.78 children, range 1-7)	*Hazard ratio (95% CI) for likelihood of parenthood* Radiotherapy to abdomen without pelvis vs. above diaphragm: HR 0.87 (0.65-1.16); Radiotherapy to pelvis vs. above diaphragm: HR 0.66 (0.48-0.90)	SB: low AB: low DB: unclear CF: low
	Reulen 2009	5133 CCS	>5 yr	Not reported	2998/4113 (72.9%) singleton pregnancies resulted in a live birth	*Odds ratio (95% CI) for likelihood of live birth* Radiotherapy to abdomen vs. no radiotherapy: 0.7 (0.5-1.0)	SB: low AB: low DB: unclear CF: low
GRADE assessment:							

Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/3, low in 2/3; Attrition bias low in 3/3; Detection bias unclear in 3/3; Confounding low in 3/3
Consistency:	0	No important inconsistency, all studies showed significant effect of radiotherapy to the ovaries
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	0	No important imprecision, high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⨁⨁⨁⊖ MODERATE
Conclusion:		Decreased likelihood of pregnancy and live birth after (increasing doses of) radiotherapy to volumes exposing the ovaries in female cancer survivors diagnosed before age 25 years. (3 studies significant effect, 10,749 participants, 3,226 events, 3 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

14. What is the likelihood of a pregnancy/live birth among female cancer patients diagnosed before age 25 years who will be treated with radiotherapy to the hypothalamic-pituitary axis?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
14.1 Likelihood pregnancy and live birth after radiotherapy to the hypothalamic-pituitary axis (n=2 studies)	Green 2009	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Radiotherapy to the hypothalamic-pituitary axis 10.0-30.0 Gy vs. ≤ 10.0 Gy: RR 0.85 (0.72-1.01); Radiotherapy to the hypothalamic-pituitary axis >30.0 Gy vs. ≤ 10.0 Gy: RR 0.61 (0.44-0.83)	SB: high risk AB: low risk DB: unclear CF: low risk
	Reulen 2009	5133 CCS	>5 yr	Not reported	2998/4113 (72.9%) singleton pregnancies resulted in a live birth	*Odds ratio (95% CI) for likelihood of live birth* Cranial radiotherapy vs. no radiotherapy: 1.1 (0.8-1.4)	SB: low AB: low DB: unclear CF: low

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/2, low in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	-1	Some inconsistency, one study showed significant effect of CRT and one study showed no significant effect.
Directness:	0	Results are direct, population and outcomes broadly generalizable

Precision:	0	No important imprecision, high total number of included patients and events and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		Decreased likelihood of pregnancy after radiotherapy to the hypothalamic-pituitary axis in female cancer survivors diagnosed before age 25 years. (1 study significant effect, 10,282 participants, 2998 events, 2 multivariable analyses)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

15. What is the likelihood of a pregnancy/live birth among female cancer patients diagnosed before age 25 years who will be treated with oophoropexy?

Outcome	Study	No. of participants	Follow up (median/mean, range) yr	Alkylating agents Radiotherapy	Events	Effect size	Risk of bias
15.1 Likelihood pregnancy and live birth after oophoropexy (n=1 study)	Green 2009	5149 CCS	>5 yr	Alkylating agents: 45.2%; Radiotherapy to ovaries/uterus: 67.6%; Cranial radiotherapy: 67.6%	Number of pregnancies not reported	*Relative risk (95% CI) for likelihood of reporting first pregnancy* Oophoropexy yes vs. no: RR 0.80 (0.58-1.09)	SB: high risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding low in 1/1
Consistency:	0	Not applicable (one study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision, only one study, but a high total number of included patients and narrow confidence intervals
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	Unclear if dose-response relationship
Plausible confounding:	0	No plausible confounding
Quality of evidence:		⊕⊕⊖⊖ LOW
Conclusion:		No significant effect of oophoropexy on the likelihood of pregnancy in female cancer survivors diagnosed before age 25 years. (1 study no significant effect, 5,149 participants, unclear number of events, 1 multivariable analysis)

Abbreviations: AB, attrition bias; CCS, childhood cancer survivors; CF, confounding; DB, detection bias; SB, selection bias; yr, year.

What female reproductive preservation methods are appropriate to offer in counselling?

1.1. In female patients (pre pubertal and postpubertal) diagnosed with cancer before 25 years who had ovarian tissue cryopreservation, is there evidence for live births after transplantation of ovarian tissue?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Ovarian tissue cryopreservation	Live births	Risk of bias
1.1 Live births after OTC (n=7 studies)	Biasin 2015	37/47 (78.7%) females with malignant disease (various)	Median 11.1 (0-17.5) yrs	Median 6.5(0.3-13.7) yrs	47 patients laparoscopic OTC *Transplantation* 1/47 autologous orthotopic ovarian tissue transplantation (from patient diagnosed with thalassemia)	*Live births* 1/1 (100%) transplanted thalassemia patient had a healthy live birth *Pregnancies* 1/1 (100%) spontaneous pregnancy	SB: unclear AB: low risk DB: unclear
	Dolmans 2013	391/476 (82%) females with malignant disease (various)	Mean age at OTC 23.0±8.5 yrs (9-39 years)	NM	476 patients laparoscopic OTC *Transplantation* 11/476 ovarian tissue transplantations (7/11 in malignant disease patients)	*Live births* 5/11 (45%) transplanted patients had healthy live births 1/11 (9%) transplanted patients had ongoing pregnancy *Pregnancies* 4/6 (66%) spontaneous pregnancies 2/6 (33%) pregnancies after in vitro fertilization (Unclear if the live births are from malignant disease patients)	SB: unclear AB: low risk DB: unclear
	Jensen 2017	140/176 (79%) females with malignant disease (various)	Age <18 years at OTC	NM	176 patients laparoscopic oophorectomy OTC *Transplantation* Unclear	2 healthy live births (from AML and HL patients) 1 induced abortion (from HL patient)	SB: low risk AB: high risk DB: unclear
	Wallace 2014	20 females with malignant disease (various)	<18 years at diagnosis	6.0 years (IQR 3.5–14.9)	20 patients OTC (18 laparoscopic, 2 oophorectomies) *Transplantation*	*Live births* 1 non-transplanted patient had 1 live birth (from Ewing sarcoma patient)	SB: high risk AB: high risk DB: unclear

				NM	*Pregnancies* 1 spontaneous pregnancy	
	Jadoul 2017	397/545 (73%) females with malignant disease (various)	Mean age at OTC 22.3±8.8 years (6 months - 39 years) 157/545 females with age <18 years at OTC	NM	545 patients laparoscopic OTC *Transplantation* 21/545 ovarian tissue transplantations 19/21 patients with malignant indications for OTC	*Live births* 7/21 (33%) transplanted patients had 10 healthy live births	SB: high risk AB: low risk DB: unclear
	Tanbo 2015	164 females with malignant disease (80%) and non-malignant disease (20%)	<25 years at OTC for patients with systematic disease; <35 years at OTC for patients with localized tumour	NM	164 patients OTC (mostly unilateral oophorectomies; in few patients laparoscopic) *Transplantation* 2/2 ovarian tissue transplantations (in malignant disease patients)	*Live births* 2/2 (100%) transplanted patients had 2 healthy live births (from T-cell lymphoma and HL patients diagnosed <25 years) (1 spontaneous pregnancy and 1 pregnancy with assisted reproduction due to concomitant male factor)	SB: low risk AB: low risk DB: unclear
	Silber 2018	108 females with malignant disease (61%) and non-malignant disease (39%)	Median age at OTC: 24 yrs (range 6-35)	NM Age at follow-up range 25-36 yrs	108 patients OTC (minilaparotomy) *Transplantation* 13/108 (12.0%) ovarian cortex transplantation; 10 (76.9%) <age 25 years at time of freezing; 8 (61.5%) malignant diagnosis	*Live births* Among females with a malignant diagnosis before age 25 years: 5/8 (62.5%) transplatned patients had 9 live births from spontaneous pregnancies	SB: low risk AB: low risk DB: unclear

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias low in 3/7, high in 2/7, unclear in 2/7; Attrition bias low in 5/7, high in 2/7; Detection bias unclear in 7/7
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness, patients without cancer diagnosis
Precision:	-1	Some imprecision, small number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality of evidence	⊕⊖⊖⊖	VERY LOW

Conclusion:	Live births after transplantation of cryopreserved ovarian tissue *(4 studies; 19 live births out of 42 transplantations (45%)*)*

Abbreviations: OTC, ovarian tissue cryopreservation; NM, not mentioned; yrs: years; HL, Hodgkin Lymphoma; AML, acute myeloid leukaemia; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

* This included also transplantations not clear if in cancer patients

1.2. In female patients (pre pubertal and postpubertal) diagnosed with cancer before 25 years, is there evidence for live births after in vitro maturation?

No studies investigating live births after in vitro maturation.

2. In female patients (pre pubertal and postpubertal) diagnosed with cancer before 25 years who had ovarian tissue cryopreservation, is there evidence for restoration of ovarian function after transplantation of ovarian tissue?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Ovarian tissue cryopreservation	Restoration ovarian function	Risk of bias
1.1 Restoration of ovarian function after OTC (n=2 studies)	Silber 2018	108 females with malignant disease (61%) and non-malignant disease (39%)	Median age at OTC: 24 yrs (range 6-35)	NM Age at follow-up range 25-36 yrs	108 patients OTC (minilaparotomy) *Transplantation* 13/108 (12.0%) ovarian cortex transplantation; 10 (76.9%) <age 25 years at time of freezing; 8 (61.5%) malignant diagnosis	*Ovarian function after transplantation* 13/13 (100%) had return of ovarian function from 4-5 months after transplantation; 8/13 (61.5%) grafts were still functioning from 28-62 months after surgery; 5/13 (38.5%) grafts ceased functioning from 22-51 months	SB: low risk AB: low risk DB: unclear
	Poirot 2019	418 females with malignant disease (75%) and non-malignant disease (25%) below age 15 yrs	Median age at OTC: 6.9 yrs (range 0.3-15) 66.5% <10 yrs 35.9% <5 yrs	NM	418 patients OTC (majority laparoscopic and entire ovary removed) *Transplantation* 3/418 (0.7%) ovarian cortex transplantation	*Ovarian function after transplantation* 1 non-cancer patient spontaneous induction of puberty; 1 neuroblastoma patient no recovery of ovarian function; 1 sickle cell disease patient recently transplated; results awaiting	SB: low risk AB: low risk DB: unclear
GRADE assessment: Study design:	+4	Observational studies					

Study limitations:	-1	Some limitations: Selection bias low in 2/2; Attrition bias low in 2/2, Detection bias unclear in 2/2
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness, patients without cancer diagnosis
Precision:	-1	Some imprecision, small number of events
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response
Plausible confounding:	0	No plausible confounding
Quality of evidence	⊕⊖⊖⊖ VERY LOW	
Conclusion:	Restoration of ovarian function after transplantation of cryopreserved ovarian tissue in post-pubertal females *(1 study; 8 restoration of ovarian function out of 13 transplantations (61.5%)*)* Induction of puberty in 1 pre-pubertal non-cancer patient after transplantation of cryopreserved ovarian tissue *(1 study; 1 induction of puberty)*	

* This included also transplantations not clear if in cancer patients

3.1. In female patients (pre pubertal and postpubertal) diagnosed with cancer before 25 years, what is the risk of Premature Ovarian Insufficiency (POI) after Oophoropexy?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Oophoropexy	Premature Ovarian Insufficiency	Risk of bias
3.1 POI after oophoropexy (n=3 studies)	Morice 1998	37 females with pelvic malignancies Group 1: 27 clear cell adenocarcinoma of the vagina and/or cervix Group 2: 9 ovarian pure dysgerminoma; 1 para-uterine soft tissue sarcoma	20.7 years (SEM 2.8) (range 7-32)	Minimum 2 years after complete remission	37 patients oophoropexy (24 by laparotomy and 13 by laparoscopy)	*Menstrual disorders* Group 1: 9/27 (33.3%) - 5 (18.5%) amenorrhea - 4 (14.8%) oligomenorrhea (unusual long interval between menstrual periods >50days) Group 2: 1/10 (10%) - 1 oligomenorrhea with normal biological tests (gonadotrophin and oestradiol levels); menstrual cycles normalized a few months after end of irradiation	SB: high risk AB: low risk DB: unclear CF: NA
	Chemaitilly 2017	921 childhood cancer survivors	NM 0-18 years	Median 24.0 (range 10.2-48.1) years after cancer diagnosis	58/921 (6.3%) patients oophoropexy	*POI* Unclear how many patients with oophoropexy had POI *Hazard ratio (95% CI) for POI* Oophoropexy yes vs. no: HR 1.33 (0.70-2.53) (in model with	SB: high risk AB: low risk DB: unclear CF: low risk

						separate treatment modalities); HR 0.72 (0.42-1.23) (in model with combining treatment modalities)	
	Fernandez-Pineda 2018	49 Hodgkin's Lymphoma female survivors Controls: 41 Hodgkin's Lymphoma female survivors without oophoropexy	Median 15 (range 4-19) years Controls Age at diagnosis Median 16(range 6-22) years	NR Age at questionnaire: 38(25-51) years Controls: Age at questionnaire: 39(26-60) years	49 patients oophoropexy	*Hazard ratio (95% CI) for POI* Oophoropexy yes vs. no: HR 0.6 (0.2-1.9) (in model adjusting for age at diagnosis); HR 1.1 (0.5-2.7) (subanalysis in survivors who received lower CED <12,000 mg/m2 and in model adjusting for age at diagnosis)	SB: low risk AB: low risk DB: unclear CF: low risk
GRADE assessment:							
Study design:	+4	Observational studies					
Study limitations:	-1	Some limitations: Selection bias high in 2/3, low in 1/3; Attrition bias low in 3/3; Detection bias unclear 3/3; Confounding low 2/3, NA in 1/3					
Consistency:	0	No important inconsistency					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision, low number of events and the confidence interval crossed the clinical decision threshold					
Publication bias:	0	Unlikely					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose -response					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:	⊕⊕⊖⊖ LOW						
Conclusion:	*No significant effect of oophoropexy on the risk of premature ovarian insufficiency* *(2 studies non-significant; 1 study no statistical testing; 1048 patients; at least 5 events)*						

Abbreviations: NM, not mentioned; NA, not applicable; SEM, standard error of mean; POI, premature ovarian insufficiency; HL, Hodgkin Lymphoma; AML, acute myeloid leukaemia; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

3.2. In female patients (pre pubertal and postpubertal) diagnosed with cancer before 25 years, is there evidence for live births after Oophoropexy?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Oophoropexy	Live births	Risk of bias
3.2 Live births after oophoropexy (n=2 studies)	Morice 1998	37 females with pelvic malignancies Group 1: 27 clear cell adenocarcinoma of the vagina and/or cervix	20.7 years (SEM 2.8) (range 7-32)	Minimum 2 years after complete remission	37 patients oophoropexy (24 by laparotomy and 13 by laparoscopy)	13/18 (72%) pregnant females delivered 15 live births (no fetal malformations related to maternal history) 5/18 (28%) pregnant females had miscarriages	SB: high risk AB: low risk DB: unclear CF: NA

		Group 2: 9 ovarian pure dysgerminoma; 1 para-uterine soft tissue sarcoma					
	Fernandez-Pineda 2018	49 Hodgkin's Lymphoma female survivors Controls: 41 Hodgkin's Lymphoma female survivors without oophoropexy	Median 15 (range 4-19) years Controls Age at diagnosis Median 16(range 6-22) years	NR Age at questionnaire: 38(25-51) years Controls: Age at questionnaire: 39(26-60) years	49 patients oophoropexy	30/49(61%) survivors with at least one pregnancy 27/30(90%) pregnant females delivered a live birth at least once No difference between probability of a first pregnancy or live birth before age 40 between OT group (p=0.1360) vs. non-OT group (p=0.4970)	SB: low risk AB: low risk DB: unclear CF: low risk

GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias high in ½, low in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 1/2, NA in 1/2
Consistency:	0	No important inconsistency
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Some imprecision
Publication bias:	-1	Some publication bias, studies with negative results are likely to not be published
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*Live births after oophoropexy* *(2 studies; 13 out of 18 pregnant patients delivered 15 live births and 27 out of 30 pregnant patients delivered at least 27 live births)* *No significant difference between probability of a first pregnancy or live birth before age 40 between OT group vs. non-OT group (1 study; 90 patients)*	

Abbreviations: NA, not applicable; SEM, standard error of mean; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

4.1. In female patients (postpubertal) diagnosed with cancer before 25 years, what is the live births outcome after embryo cryopreservation?

No studies investigating live births after *embryo cryopreservation*

4.2. In female patients (postpubertal) diagnosed with cancer before 25 years, what is the live births outcome after oocyte cryopreservation?

No studies investigating live births after oocyte cryopreservation

5.1. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is the risk of Premature Ovarian Insufficiency after Gonadotropin-releasing hormone (GnRH) analogues during cancer treatment?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Gonadotropin-releasing hormone (GnRH) analogues	Premature Ovarian Insufficiency	Risk of bias
5.1. Premature ovarian Insufficiency after GnRH analogues (n=2 studies)	Pereyra 2001	*Study group* 12 postmenarchal females - Subgroup 1: 5 treated with CT before BMT - Subgroup 2: 7 treated with CT and supradiaphragmatic irradiation but no BMT *Control group 1:* 5 premenarchal females previously treated with CT *Control group 2*: 4 postmenarchal females previously treated with CT and BMT	*Study group:* 14.7-20 yrs *Control group 1*: 3- 7.5 yrs *Control group 2:* 15.9- 20 yrs	*Study group:* up to 5 yrs (mean or range NM) *Control group 1:* 18 yrs *Control group 2:* 6 yrs	12 patients GnRH analogue during CT (in study group) (3.75 mg im depot monthly until 30 days after CT)	*Menstrual disorders* Study group: 0/12 (0%) amenorrhea Control group 1: 1/5 (20%) oligomenorrhea Control group 2: 4/4 (100%) hypergonadotrophic hypoestrogenic amenorrhea 4/4 hormone replacement therapy	SB: high risk AB: high risk DB: unclear CF: high risk
	Meli 2018	36 adolescent females treated with CT	Median 14 yrs (range 10-18)	Median 5 yrs (range 1-17) since end of treatment	36 patients GnRH analogue during CT (monthly depot im injection of 3.75 mg GnRH-a (Decapeptyl) or a triple dose of GnRH-a (11.25 mg) every 3 months)	Menstrual cycles in 1st year after therapy: 24 (66%) regular menstrual cycles 7 (19%) oligomenorrhea 5 (14%) amenorrhea Menstrual cycles/sexual hormone levels at last follow-up: 29 (81%) regular menstrual cycle 3 (8%) oligomenorrhea 4 (11%) amenorrhea	SB: unclear AB: low risk DB: unclear CF: high risk

			In 4/9 (44%) treated with HSCT and high-doses of alkylating agents ovarian function was not preserved
GRADE assessment:			
Study design:	+4	Observational study	
Study limitations:	-2	Important limitations: Selection bias high in 1/2, unclear in 1/2; Attrition bias high in 1/1, low in 1/1; Detection bias unclear in 2/2; Confounding high in 2/2	
Consistency:	0	No important inconsistency	
Directness:	0	Results are direct, population and outcomes broadly generalizable	
Precision:	-1	Important imprecision, small study sample	
Publication bias:	0	Unlikely	
Effect size:	0	No large magnitude of effect	
Dose-response:	0	No dose-response	
Plausible confounding:	0	No plausible confounding	
Quality of evidence:	⊕⊖⊖⊖ VERY LOW		
Conclusion:	*Fewer patients had amenorrhea after GnRH analogues during cancer treatment compared to patients without GnRH analogues during cancer treatment (1 study without statistical testing; 0 out of 12 patients with GnRH had amenorrhea, 4 out of 9 patients without GnRH had amenorrhea)* *Majority of females had regular menstrual cycles 1 to 17 years after end of alkylating agent chemotherapy and GnRH analogues (2 studies without statistical testing; 4 out of 48 patients with GnRH had amenorrhea all of whom were treated with HSCT and high-doses of alkylating agents)*		

Abbreviations: N/A, not applicable; NM, not mentioned; CT, chemotherapy; BMT: bone marrow transplant; GnRH: gonadotropin releasing hormone; im: intramuscular; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

5.2. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, is there evidence for live births after Gonadotropin-releasing hormone (GnRH) analogues during cancer treatment?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Gonadotropin-releasing hormone (GnRH) analogues	Live births	Risk of bias
5.2. Live births after GnRH analogues (n=2 studies)	Pereyra 2001	*Study group* 12 postmenarchal females - Subgroup 1: 5 treated with CT before BMT - Subgroup 2: 7 treated with CT and supradiaphragmatic irradiation but no BMT	*Study group 1:* 14.7-20 yrs *Control group 1*: 3- 7.5 yrs *Control group 2:* 15.9- 20 yrs	*Study group:* up to 5 yrs (mean or range NM) *Control group 1*: 18 yrs *Control group 2*: 6 yrs	12 patients GnRH analog during CT (in study group) (3.75 mg im depot monthly until 30 days after CT)	*Study group - Subgroup 1:* 2/2 (100%) pregnant females delivered 3 healthy live births *Control group 1:* 3/3 (100%) pregnant females delivered 5 healthy live births *Control group 2:* No pregnancies	SB: high risk AB: high risk DB: unclear CF: high risk

		Control group 1: 5 premenarchal females previously treated with CT *Control group 2*: 4 postmenarchal females previously treated with CT and BMT					
	Meli 2018	36 adolescent females treated with CT	Median 14 yrs (range 10-18)	Median 5 yrs (range 1-17) since end of treatment	36 patients GnRH analogue during CT (monthly depot im injection of 3.75 mg GnRH-a (Decapeptyl) or a triple dose of GnRH-a (11.25 mg) every 3 months)	5/5 (100%) pregnant females delivered 8 healthy live births	SB: unclear AB: low risk DB: unclear CF: high risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-2	Important limitations: Selection bias high in 1/2, unclear in 1/2; Attrition bias high in 1/1, low in 1/1; Detection bias unclear in 2/2; Confounding high in 2/2
Consistency:	0	No important inconsistency
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Important imprecision, small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*Live births in patients treated with and without GnRH analogues during cancer treatment* *(2 studies without statistical testing; 7 out of 7 pregnant patients with GnRH delivered 11 live births, 3 out of 3 pregnant patients without GnRH delivered 5 live births)*	

Abbreviations: N/A, not applicable; NM, not mentioned; CT, chemotherapy; BMT: bone marrow transplant; GnRH: gonadotropin releasing hormone; im: intramuscular; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

5.3. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is risk of Premature Ovarian Insufficiency (POI) after Immunomodulators AS101, S1P?

No studies investigated effect after immunomodulators AS101, S1P.

5.4. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is the risk of Premature Ovarian Insufficiency (POI) after Oral contraceptive pill during cancer treatment?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Oral contraceptive pill	Premature Ovarian Insufficiency	Risk of bias
5.4 POI after oral contraceptive pill (n=1 study)	Longhi 2003	Study group: 31 females with osteosarcoma 24/31 postpubertal 7/31 prepubertal Control group: 90 females with osteosarcoma 68/90 postpubertal 22/90 prepubertal	Study group: mean19.4 years (4-40) Control group: mean 16.8 years (7-43)	Study group: Mean 29.4 (9-43) months post-CT Control group: NM	Study group: OC (desogestrel 0.150 mg + etinilestradiol 0.020 mg) given continuously during neo-adjuvant CT (duration about 36 weeks) 19/24 received OC; 5/24 received no OC (3 analyzed in control group) Control group: no OC	*Permanent amenorrhea post CT* Study group: 3/24 (13%) Control group: 3/71 (4%)	SB: High risk AB: Low risk DB: Unclear CF: High risk

GRADE assessment:		
Study design:	+4	Observational study study (Single centre retrospective cohort)
Study limitations:	-1	Some limitations: Selection bias high in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding high in 1/1
Consistency:	0	N/A (1 study)
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-2	Important imprecision, only 1 study included with small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*More patients had amenorrhea after oral contraceptive pill during chemotherapy compared to patients without oral contraceptive pill during chemotherapy*	

	(1 study without statistical testing; 3 out of 24 patients with oral contraceptive pill had amenorrhea, 3 out of 71 patients without oral contraceptive pill had amenorrhea)

Abbreviations: NM, not mentioned; NA, not applicable; FU, follow-up; CT, chemotherapy; OC, oral contraceptive; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

6. In female patients diagnosed with cancer before age 25 years who underwent fertility preservation, what is the evidence of patient-related (long-term and short-term) complications and the offspring-related complications?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Ovarian tissue cryopreservation	Complications	Risk of bias
	Procedural complications and dely of treatment after ovarian tissue collection						
6.1. Complications after OTC (n=10 studies)	Babayev 2013	21/28 (75%) females with malignant disease (various)	Mean 13.9±1.5 (2.3-20.9) years	Mean 5.2±0.8 years after OTC	28 patients laparoscopic OTC *Transplantation* 0/28 ovarian tissue transplantations	0/28 Complications (minimal or none blood loss)	SB: unclear AB: low risk DB: unclear
	Biasin 2015	38/47 (80.8%) females with malignant disease (various)	Median 11.1 (0-17.5) years	Median 6.5(0.3-13.7) years	47 patients laparoscopic OTC *Transplantation* 1/47 autologous orthotopic ovarian tissue transplantation	0/47 Acute or chronic complications	SB: unclear AB: low risk DB: unclear
	Chambon 2016	28/36 (77.8%) females with malignant disease (various)	<20 years old at diagnosis	Median 36 (1-112) months after harvest	36 patients laparoscopic OTC *Transplantation* 0/36 ovarian tissue transplantations	1/36 Post OTC bleeding (in patient with sickle cell disease and protein S deficiency) 1/36 Delay of treatment (CT)	SB: unclear AB: low risk DB: unclear
	Dolmans 2013	391/476 (82%) females with malignant disease (various)	Mean age at OTC 23.0±8.5 years (9-39 years)	NM	476 patients laparoscopic OTC *Transplantation* 11/476 ovarian tissue transplantations (7/11 in malignant disease patients)	0/476 Serious postoperative or long-term complications No delay of treatment	SB: unclear AB: low risk DB: unclear
	Lima 2014	48/54(89%) females with malignant	Mean age at ovarian harvest	48h	54 patients laparoscopic OTC	1/54 Intraoperative complication: bleeding	SB: low risk AB: low risk

	disease (various)	160.9±6.9 months		*Transplantation* 0/54 ovarian tissue transplantations	requiring red blood cell transfusion 0/54 Postoperative or long-term complications No delay of the oncological treatment	DB: unclear
Poirot 2007	47 females with malignant disease(various) 20/47(43%) Metastatic neuroblastoma	NM Prepubertal	Median 30 (10-60) months	47 patients laparoscopic (40) and minilaparotomy (7) OTC *Transplantation* 0/47 ovarian tissue transplantations	0/47 Postoperative complications No delay of oncological treatment	SB: low risk AB: low risk DB: unclear
Wallace 2014	20 females with malignant disease (various)	<18 years old at diagnosis	6.0 years (IQR 3.5-14.9)	20 patients OTC (18 laparoscopic, 2 oophorectomies) *Transplantation* NM	0/20 Complications 21 Patients consented to OTC but in 1/21 procedure failed due to technical problems with surgical equipment: no adverse effect on the patient	SB: high risk AB: high risk DB: unclear
Jadoul 2017	397/545 (73%) females with malignant disease (various)	Mean age at OTC 22.3±8.8 years (6 months - 39 years) 157/545 females with age <18 years at OTC	NM	545 patients laparoscopic OTC *Transplantation* 21/545 ovarian tissue transplantations 19/21 patients with malignant indications for OTC	5/140 Minor complications (raised temperature, labial hematoma, urinary infection, bowel irritation and psychological distress) 1/140 Major complication (patient had second laparoscopy for intra-abdominal hemorrhage due to ovarian biopsy) (Complications reported by questionnaire in 140 patients)	SB: high risk AB: low risk DB: unclear
Rowell 2019	64 females (83%	Median age at	41 (64.1%)	64 patients OTC	No intraoperative	SB: low risk

		malignant disease)	OTC 12 yrs (range 0.4-23)	patients are ≥1 yrs from time of OTC	(majority laparasopic unilateral oophorectomy)	complications related to the laparoscopic oophorectomy occurred Median estimated blood loss of patients undergoing OTC, without primary mass excision: 3 ml No reported 30-day postoperative complications Median time from operation to initiation of medical therapy: 6 days with no unanticipated delays in treatment initiation	AB: low risk DB: unclear
	Transplantation / Contamination of malignant cells in tissue						
	Tanbo 2015	164 females with malignant disease (80%) and non-malignant disease (20%)	NM <25 years at OTC for patients with systematic disease; <35 years at OTC for patients with localized tumour	NM OTC during 11 years	164 patients OTC (mostly unilateral oophorectomies; in few patients laparoscopic) *Transplantation* 2/2 ovarian tissue transplantations (in malignant disease patients) 6 patients requested ovarian tissue transplantations (various malignant diagnoses)	1/2 transplanted patients had contamination of leukemic cells in cryopreserved tissue (patient diagnosed with acute lymphatic leukaemia at 22 years)	SB: low risk AB: low risk DB: unclear
	Rosendahl 2010	26 patients with leukaemia	NR Median age at OTC: 16 (2-31) years	NM Cryopreserved ovarian tissue fragments were thawed and examined. No follow-up time	37 laparoscopic or minilaparotomy OTC *Transplantation* 0/37 ovarian tissue transplantations	0/37 no malignant cells detected by histology or immunohistochemistry 6/8 (16%) patients with leukemic cells in cryopreserved tissue	SB: high risk AB: low risk DB: unclear

			reported.		detected by PCR (8 patients with a specific chromosomal abnormalities in the malignant cells which allowed detection by PCR)	
Seshadri 2006	26 female patients with Hodgkin lymphoma	Median age 22 years (13-29)	NM	26 laparoscopic OTC *Transplantation* 0/26 ovarian tissue transplantation	0/26 No evidence of Hodgkin lymphoma involvement by morphology or immunohistochemistry (95% CI for ' true' rate of involvement 0-11%)	SB: unclear AB: low risk DB: unclear
Biasin 2015	38/47 (80.8%) females with malignant disease (various)	Median 11.1 (0-17.5) yrs	Median 6.5(0.3-13.7) yrs	47 patients laparoscopic OTC *Transplantation* 1/47 autologous orthotopic ovarian tissue transplantation (from patient diagnosed with thalassemia)	0/47 No evidence of tumour contamination by histology examination	SB: unclear AB: low risk DB: unclear
Babayev 2013	21/28 (75%) females with malignant disease (various)	Mean 13.9±1.5 (2.3-20.9) years	Mean 5.2±0.8 years after OTC	28 patients laparoscopic OTC *Transplantation* 0/28 ovarian tissue transplantations	0/28 No evidence of tumour contamination	SB: unclear AB: low risk DB: unclear
Chambon 2016	28/36 (77.8%) females with malignant disease (various)	<20 years old at diagnosis	Median 36 (1-112) months after harvest	36 patients laparoscopic OTC *Transplantation* 0/36 ovarian tissue transplantations	0/36 No evidence of tumour contamination by histology examination	SB: unclear AB: low risk DB: unclear
Dolmans 2013	391/476 (82%) females with malignant disease (various)	NM Mean age at OTC 23.0±8.5 years (9-39 years)	NM	476 patients laparoscopic OTC *Transplantation* 11/476 ovarian tissue transplantations (7/11 in malignant	5/391 (1.3%) Evidence of tumour contamination by histology examination (in 3 leukaemia patients and 2 non-Hodgkin lymphoma patient)	SB: unclear AB: low risk DB: unclear

				disease patients)		
Dolmans 2016	48 sarcoma patients	NR Mean age at OTC: 16.3 years ±SD 7.27	NA	48 patients OTC	0/26 No evidence of tumour contamination by sensitive methods	SB: low risk AB: high risk DB: unclear
Jensen 2017	140/176 (79%) females with malignant disease (various)	NM Age <18 years at OTC	NM	176 patients laparoscopic oophorectomy OTC *Transplantation* Unclear	0/176 No evidence of tumour contamination	SB: low risk AB: high risk DB: unclear
Silber 2018	108 females with malignant disease (61%) and non-malignant disease (39%)	Median age at OTC: 24 yrs (range 6-35)	NM Age at follow-up range 25-36 yrs	108 patients OTC (minilaparotomy) *Transplantation* 13/108 (12.0%) ovarian cortex transplantation; 10 (76.9%) <age 25 years at time of freezing; 8 (61.5%) malignant diagnosis	0/13 No evidence of tumour contamination, of whom 3 leukaemia patients	SB: low risk AB: low risk DB: unclear

GRADE assessment (complications related to ovarian tissue collection)

Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias low in 3/9, high in 2/9, unclear in 4/9; Attrition bias low in 8/9, high in 1/9; Detection bias unclear in 9/9
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness, patients without cancer diagnosis
Precision:	0	No important imprecision
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding

Quality of evidence: ⊕⊕⊖⊖ LOW

Conclusion: *Three female patients with intraoperative bleeding after ovarian tissue cryopreservation (9 studies; 3 patients with complications)*

No studies investigated offspring-related complications after ovarian tissue cryopreservation.

GRADE assessment: (contamination of malignant cells in tissue)

Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias low in 3/10, high in 1/10, unclear in 5/10; Attrition bias low in 8/10, high in 2/10; Detection bias unclear in 10/10
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness, patients without cancer diagnosis
Precision:	-1	Some imprecision; low number of patients and events

Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*In 12 females with leukaemia or non-Hodgkin lymphoma the cryopreserved ovarian tissues had tumour cell contamination (4 studies; 12 patients with contamination of malignant cells)* *No evidence of tumour contamination in cryopreserved ovarian tissue in females with non-metastasized solid tumours (7 studies; 0 patients with contamination of malignant cells)* *No evidence of tumour contamination in cryopreserved ovarian tissue in females with Hodgkin lymphoma (1 study; 0 patients with contamination of malignant cells)*	

Abbreviations: NM, not mentioned; NA, not applicable; FU, follow-up; CT, chemotherapy; RT, radiotherapy; OTC, ovarian tissue cryopreservation; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding; PCR: polymerase chain reaction

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Gonadotropin-releasing hormone (GnRH) analogues	Complications	Risk of bias
6.2. Complications after GnRH analogues (n=1 study)	Meli 2018	36 adolescent females treated with CT	Median 14 yrs (range 10-18)	Median 5 yrs (range 1-17) since end of treatment	36 patients GnRH analogue during CT (monthly depot im injection of 3.75 mg GnRH-a (Decapeptyl) or a triple dose of GnRH-a (11.25 mg) every 3 months)	No late effects occurred	SB: unclear AB: low risk DB: unclear CF: high risk

GRADE assessment:		
Study design:	+4	Observational study
Study limitations:	-2	Important limitations: Selection bias unclear in 1/1; Attrition bias low in 1/1; Detection bias unclear in 1/1; Confounding high in 1/1
Consistency:	0	NA, only one study
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1	Important imprecision, small study sample
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*No long-term complications reported after GnRH analogues during cancer treatment (1 study without statistical testing; 0 out of 36 patients with GnRH reported complications)*	

Abbreviations: NA, not applicable; NM, not mentioned; CT, chemotherapy; BMT: bone marrow transplant; GnRH: gonadotropin releasing hormone; im: intramuscular; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

7. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is the association between live births and timing (before cancer treatment versus after cancer treatment)after:

- **Ovarian tissue cryopreservation?**
- **In vitro maturation ?**
- **Oophoropexy ?**

No studies investigating live births and timing after in vitro maturation.

7.1. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is the association between live births and timing (before cancer treatment versus after cancer treatment) for Ovarian tissue cryopreservation?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Ovarian tissue cryopreservation	Timing of collection and live births	Risk of bias
7.1 Timing OTC and live births (n=2 studies)	Biasin 2015	38/47 (80.8%) females with malignant disease (various)	Median 11.1 (0-17.5) yrs	Median 6.5(0.3-13.7) yrs	47 patients laparoscopic OTC *Transplantation* 1/47 autologous orthotopic ovarian tissue transplantation (from patient diagnosed with thalassemia)	*Timing of collection* 1/1 (100%) transplanted thalassemia patient had OTC before CT *Live births* 1/1 (100%) transplanted thalassemia patient had a healthy live birth	SB: unclear AB: low risk DB: unclear
	Dolmans 2013	391/476 (82%) females with malignant disease (various)	NM Mean age at OTC 23.0±8.5 yrs (9-39 years)	NM	476 patients laparoscopic OTC *Transplantation* 11/476 ovarian tissue transplantations (7/11 in malignant disease patients)	*Timing of collection* 442/476 (93%) OTC before cancer treatment 34/476 (7%) OTC after CT *Live births* 5/11(45%) transplanted patients had healthy live births 1/11(9%) transplanted patients had ongoing pregnancy (Unclear if the live births are from	SB: unclear AB: low risk DB: unclear

		malignant disease patients)
GRADE assessment:		
Study design:	+4	Observational studies
Study limitations:	-1	Some limitations: Selection bias unclear in 2/2; Attrition bias low in 2/2; Detection bias unclear in 2/2
Consistency:	0	No important inconsistency
Directness:	-1	Some indirectness, patients without cancer diagnosis
Precision:	-1	Some imprecision
Publication bias:	-1	Some publication bias, studies with negative results are likely to not be published
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose -response
Plausible confounding:	0	No plausible confounding
Quality of evidence:	⊕⊖⊖⊖ VERY LOW	
Conclusion:	*Live births after transplantation of cryopreserved ovarian tissue collected before cancer treatment (1 study; 5 patients out of 11 transplantations)*	

Abbreviations: OTC, ovarian tissue cryopreservation; NM, not mentioned; CT, chemotherapy; RT, radiotherapy; HL, Hodgkin Lymphoma; AML, acute myeloid leukaemia; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

7.2. In female patients (pre pubertal and postpubertal) diagnosed with cancer before age 25 years, what is the association between live births and timing (before cancer treatment versus after cancer treatment) for oophoropexy?

Outcome	Study	Participants	Age at diagnosis	Follow up (median/mean, range) yr	Oophoropexy	Timing of collection and live births	Risk of bias
7.2 Timing of oophoropexy and live births (n=2 studies)	Morice 1998	37 females with pelvic malignancies Group 1: 27 clear cell adenocarcinoma of the vagina and/or cervix Group 2: 9 ovarian pure dysgerminoma; 1 para-uterine soft tissue sarcoma	20.7 years (SEM 2.8) (range 7-32)	Minimum 2 years after complete remission	37 patients oophoropexy (24 by laparotomy and 13 by laparoscopy)	*Timing of collection* Oophoropexy before RT and CT 13/18 (72%) pregnant females delivered 15 live births (no fetal malformations related to maternal history) 5/18 (28%) pregnant females had miscarriages	SB: high risk AB: low risk DB: unclear

	Fernandez-Pineda 2018	49 Hodgkin's Lymphoma female survivors Controls: 41 Hodgkin's Lymphoma female survivors without oophoropexy	Median 15 (range 4-19) years Controls Age at diagnosis Median 16(range 6-22) years	NR Age at questionnaire: 38(25-51) years Controls: Age at questionnaire: 39(26-60) years	49 patients oophoropexy	30/49(61%) survivors with at least one pregnancy 27/30(90%) pregnant females delivered a live birth at least once No difference between probability of a first pregnancy or live birth before age 40 between OT group (p=0.1360) vs. non-OT group (p=0.4970)	SB: low risk AB: low risk DB: unclear
GRADE assessment:							
Study design:	+4	Observational study (retrospective analysis of a consecutive case series)					
Study limitations:	-1	Some limitations: Selection bias low in 1/1, high in 1/1; Attrition bias low in 2/2; Detection bias unclear in 2/2					
Consistency:	0	No important inconsistency					
Directness:	0	Results are direct, population and outcomes broadly generalizable					
Precision:	-1	Some imprecision					
Publication bias:	-1	Some publication bias, studies with negative results are likely to not be published					
Effect size:	0	No large magnitude of effect					
Dose-response:	0	No dose -response					
Plausible confounding:	0	No plausible confounding					
Quality of evidence:	⨁⊖⊖⊖ VERY LOW						
Conclusion:	*Live births after oophoropexy before cancer treatment* *(2 studies; 13 out of 18 pregnant patients delivered 15 live births and 27 out of 30 pregnant patients delivered at least 27 live births)*						

8. **In female patients (postpubertal) diagnosed with cancer before age 25 years, what is the association between live births and timing (before cancer treatment versus after cancer treatment) after:**
   - **Embryo cryopreservation?**
   - **Oocyte cryopreservation?**

No studies investigating live births and timing after embryo cryopreservation or oocyte cryopreservation.

9. **In female patients diagnosed with cancer before age 25 years, is there evidence for pregnancies and live births after oocyte donation?**

Outcome	Study	Participants Malignant disease	Age at diagnosis	Follow up (median/mean, range) yr	Oocyte donation	Pregnancy outcomes	Risk of bias
9. Pregnancy outcome with	Vernaeve 2007	33 female cancer survivors (various	21.0 yrs (95% CI 17.3-24.7) yr	NM	OD cycle with fresh embryo	*Pregnancy outcomes cancer survivors vs. controls*	SB: unclear AB: low risk

oocyte donation (n=2 studies)		diagnoses) Controls: 33 females without history of cancer therapy			transfer after ICSI (some patients repeated the OD procedure)	Pregnancies after OD: 19/33 (57.6%) vs. 13/33 (39.4%); p=0.1 Ongoing pregnancies after OD: 15/33 (45.4%) vs. 9/33 (27.3%); p=0.1 Delivery rate: 15/33 (45.4%) cancer survivors delivered 18 babies vs. 9/33 (27.3%) controls delivered 10 babies, p=0.1 Complications in study group: 3/15 (20%) premature delivery (<37 weeks) 1/15 (7%) placental hemorrhage with stillborn child 1/15 (7%) Pre-eclampsia Complications in control group: 1/9 (11%) premature delivery (<37 weeks)	DB: unclear CF: low risk
	Marklund 2018	31 female cancer survivors (various diagnoses) Controls: 212 females without history of cancer therapy	20.2 (range 3-38) yr	14.9 (range 2-34) yr	Cancer survivors underwent 102 egg donor treatment cycles (52 with fresh embryos and 50 with cryopreserved embryos)	*Pregnancy outcomes cancer survivors vs. controls* Cancer survivors: 25 pregnancies in 20 females Controls: 244 pregnancies in 212 females *Odds ratios (95% CI) for obstetric and perinatal outcomes in cancer survivors vs. controls adjusted for BMI and maternal age at first antenatal visit* Preeclampsia: 2.79 (1.07-7.34) Hypertensive disorders of pregnancy: 1.80 (0.69-4.69) Preterm premature rupture of membranes: 3.85 (0.96-15.42) Hemorrhage (>1000 mL): 1.22 (0.34-4.38)	SB: low risk AB: low risk DB: unclear CF: low risk

		Small for gestational age: 2.12 (0.24-18.68) Neonatal intensive care unit: 1.14 (0.36-3.61) Very preterm birth: 17.39 (3.99-75.79) Moderate preterm birth: 2.92 (0.88-9.66) APGAR <7: 2.40 (0.24-24.46)
GRADE assessment:		
Study design:	+4	Observational study (Retrospective matched controlled analysis)
Study limitations:	-1	Some limitations: Selection bias low in 1/2, unclear in 1/2; Attrition bias low in 2/2; Detection bias unclear in 2/2; Confounding low in 2/2
Consistency:	0	No important inconsistency
Directness:	0	Results are direct, population and outcomes broadly generalizable
Precision:	-1/-2	Some imprecision, with small study samples and only one study on love births
Publication bias:	0	Unlikely
Effect size:	0	No large magnitude of effect
Dose-response:	0	No dose-response
Plausible confounding:	0	No plausible confounding
Quality of evidence **Conclusion:**	⊕⊖⊖⊖ VERY LOW *Live births after oocyte donation* *(1 study; 15 out of 19 patients delivered 18 live births)* ⊕⊕⊖⊖ LOW *Pregnancy-related complications (premature delivery, placental hemorrhage with still born child, pre-eclampsia) after oocyte donation* *(2 studies 64 patients)*	

Abbreviations: N/A, not applicable; NM, not mentioned; OD, Oocyte donation; SB, selection bias; AB, attrition bias; DB, detection bias; CF, confounding

10. In female patients diagnosed with cancer before age 25 years, is there evidence for pregnancies with surrogacy (using own eggs in gestational surrogate)?

No studies investigating pregnancy-related outcomes after surrogacy (using own eggs in gestational surrogate)

11. In female patients diagnosed with cancer before age 25 years, what is the risk of medical problems in pregnancy after fertility preservation methods?

No studies investigating perinatal complications after reproductive preservation methods in patients treated with radiotherapy to volumes exposing the ovaries and/or uterus with or without estrogen supplementation