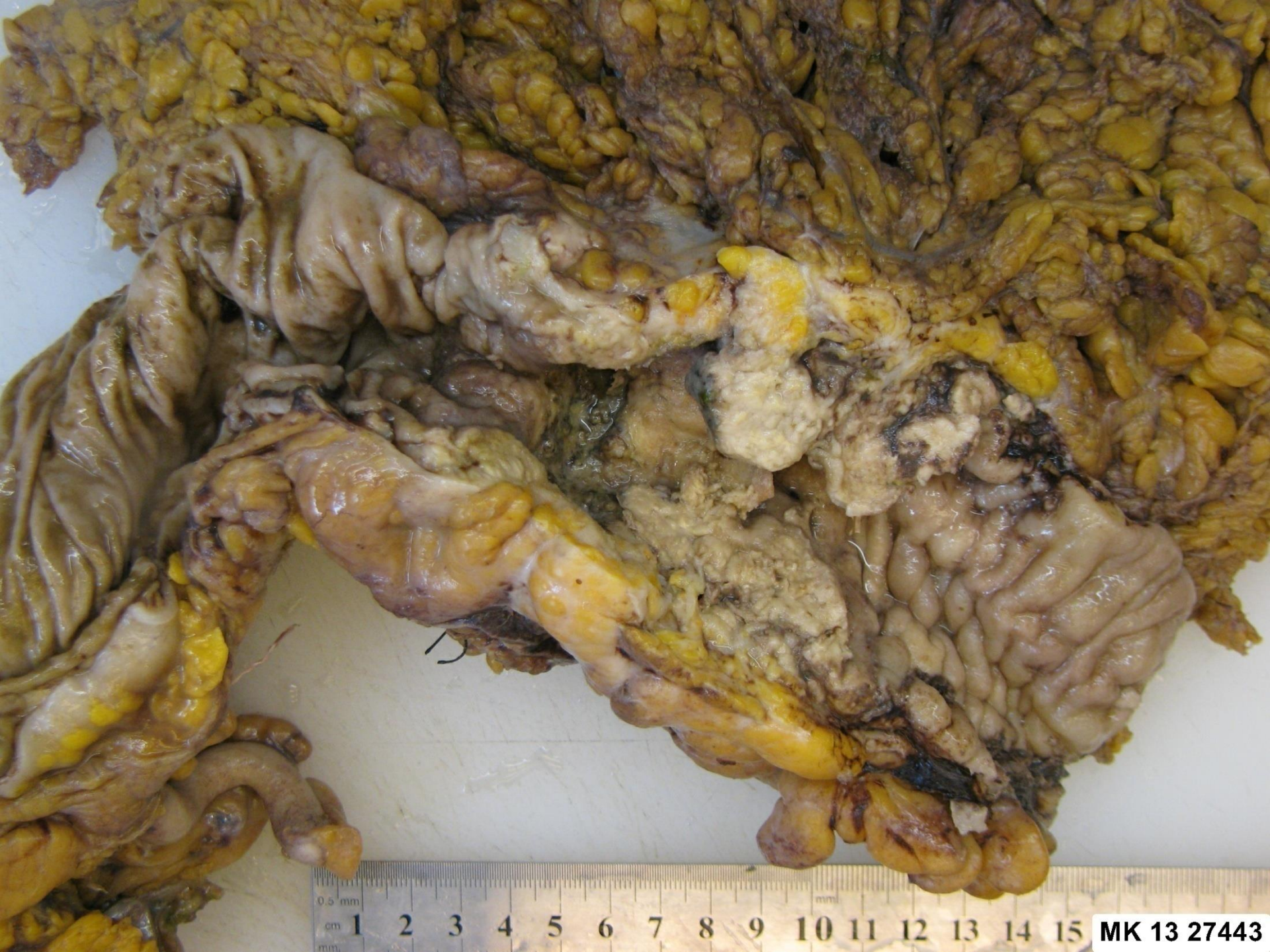
XIX. Martinský bioptický seminár SD-IAP
Lúčky , október 2013

prípad č. 498

Michal Kalman
Univerzitná nemocnica Martin

51-ročný muž, pravostranná hemicolectomia s cirkulárne rastúcim exulcerovaným tumorom v aborálnej časti resekátu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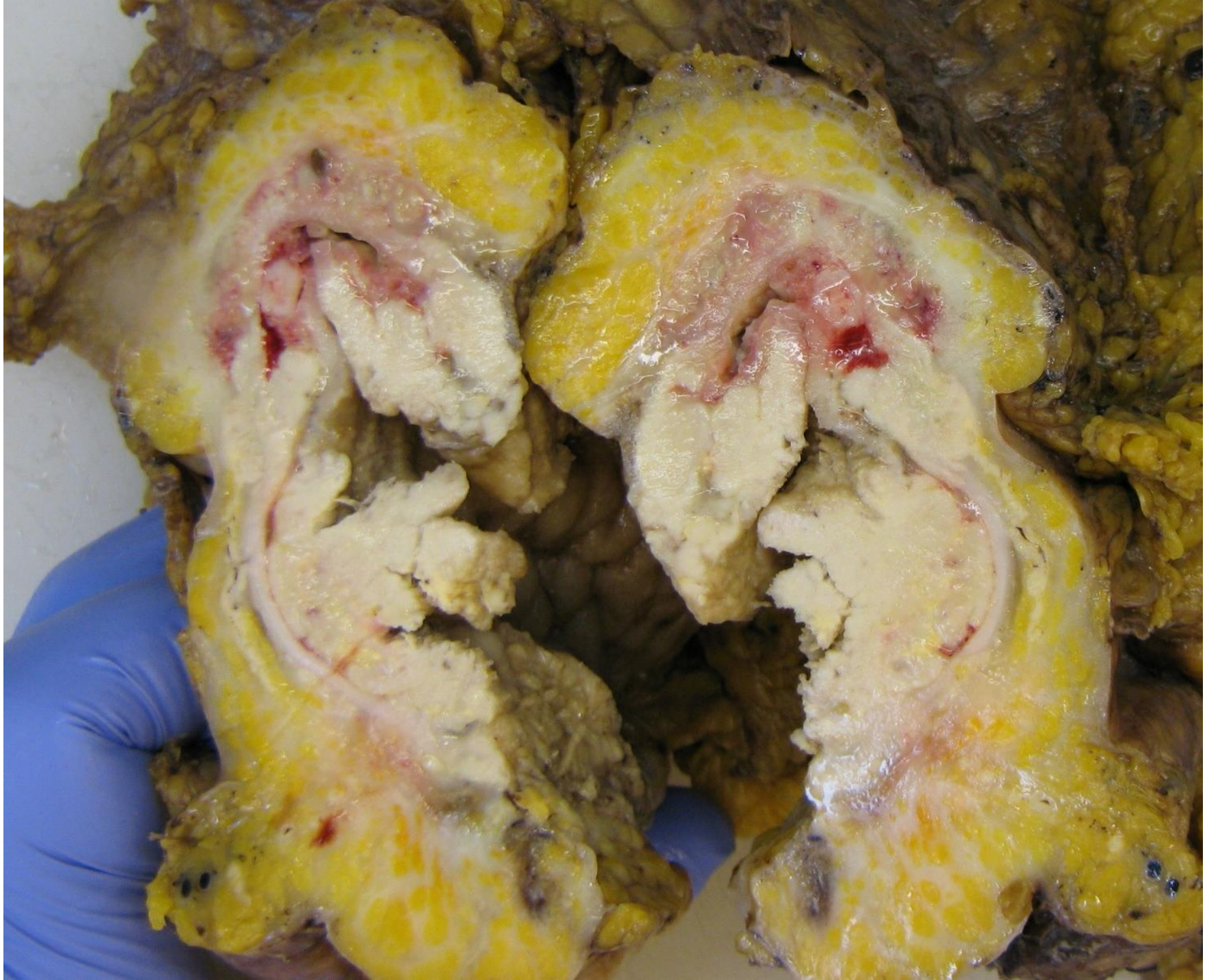
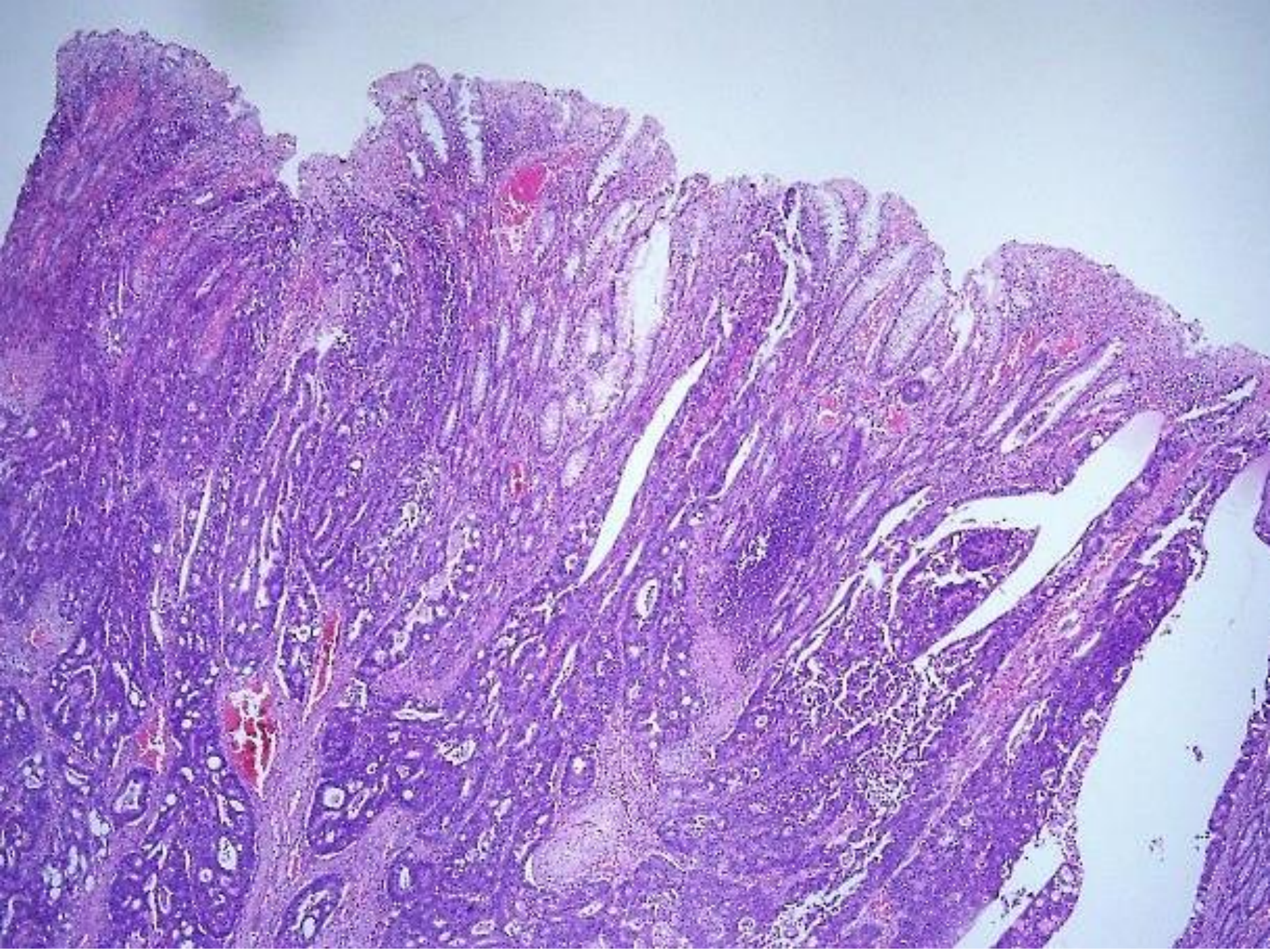
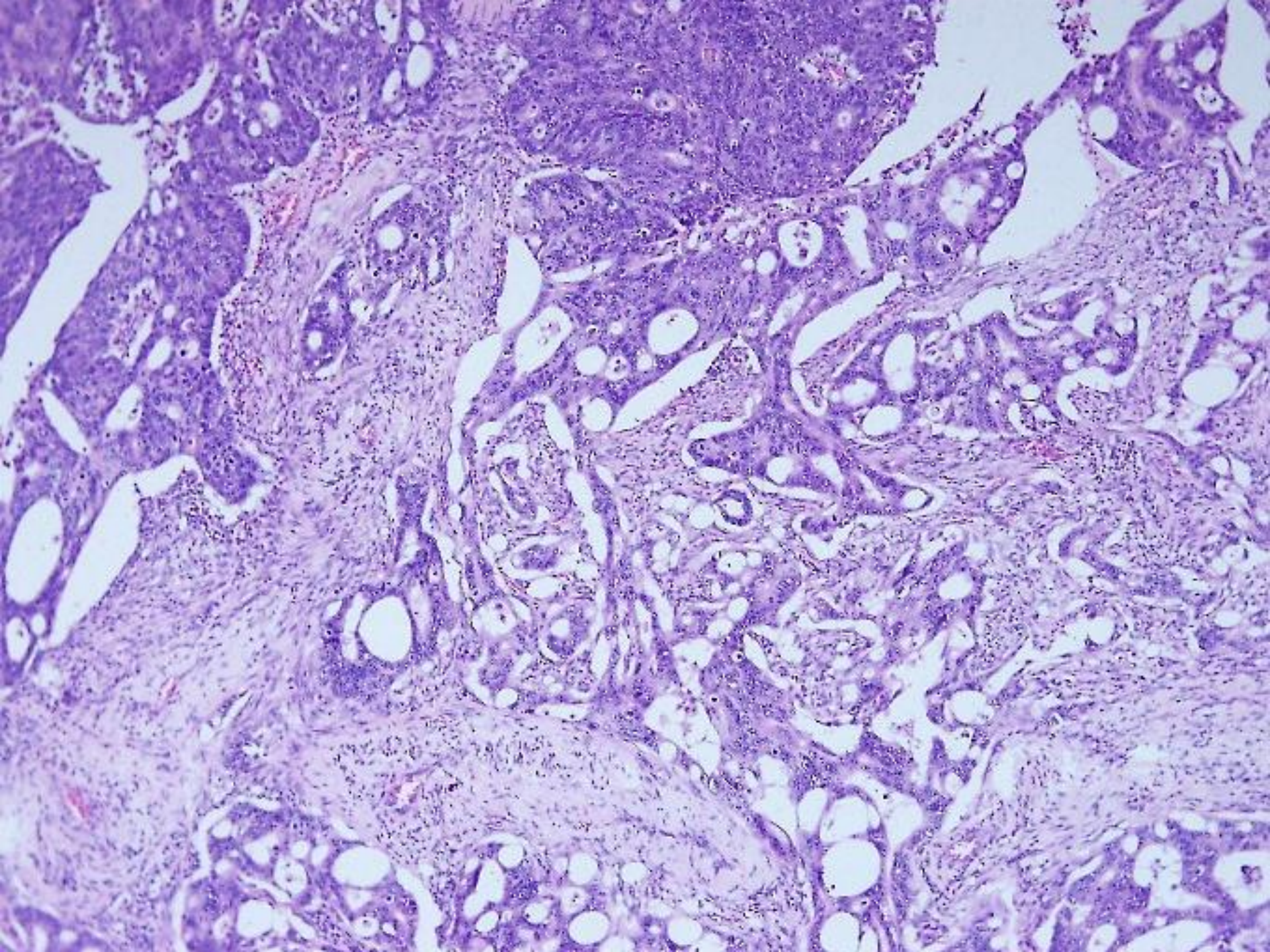
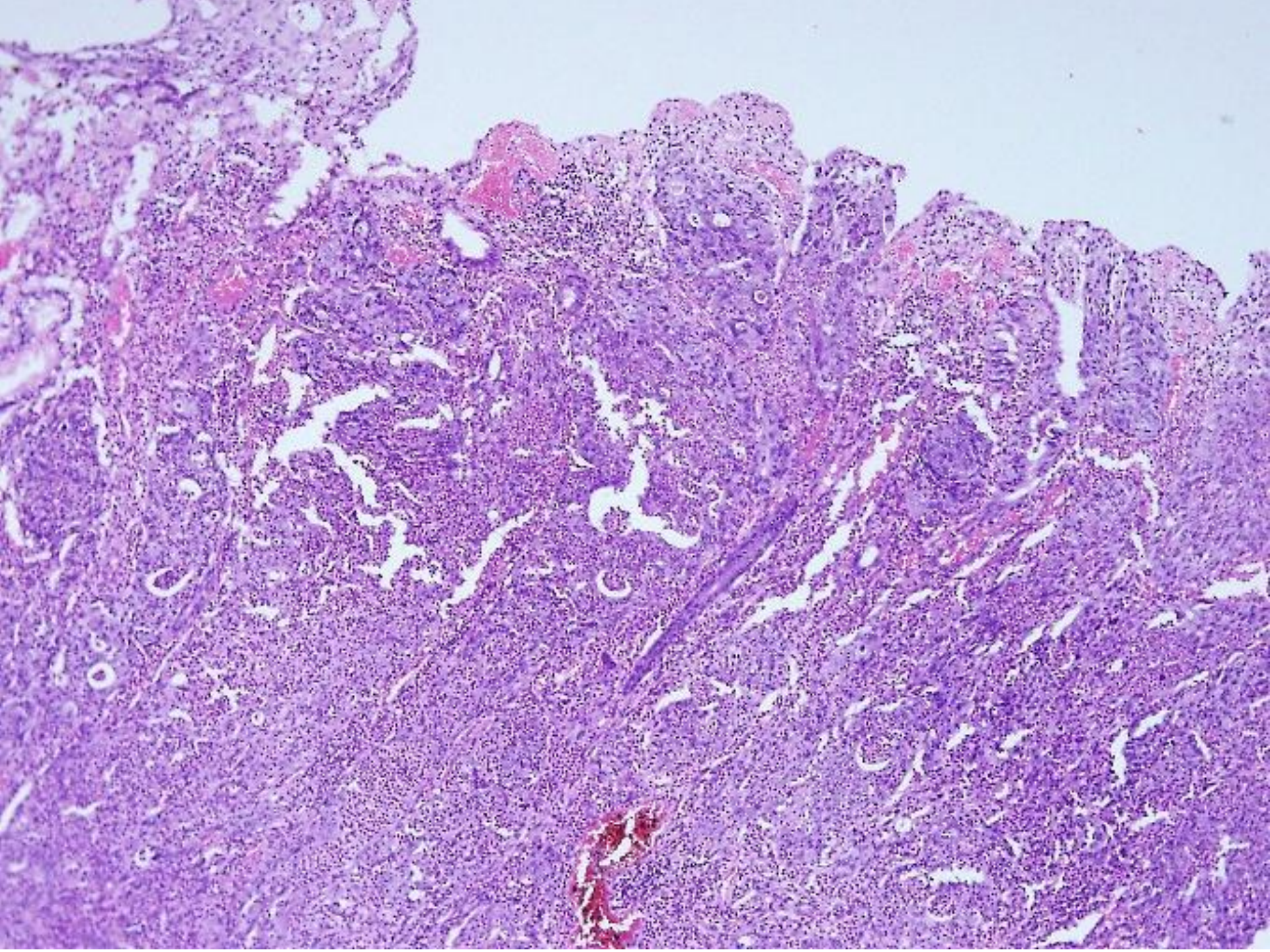
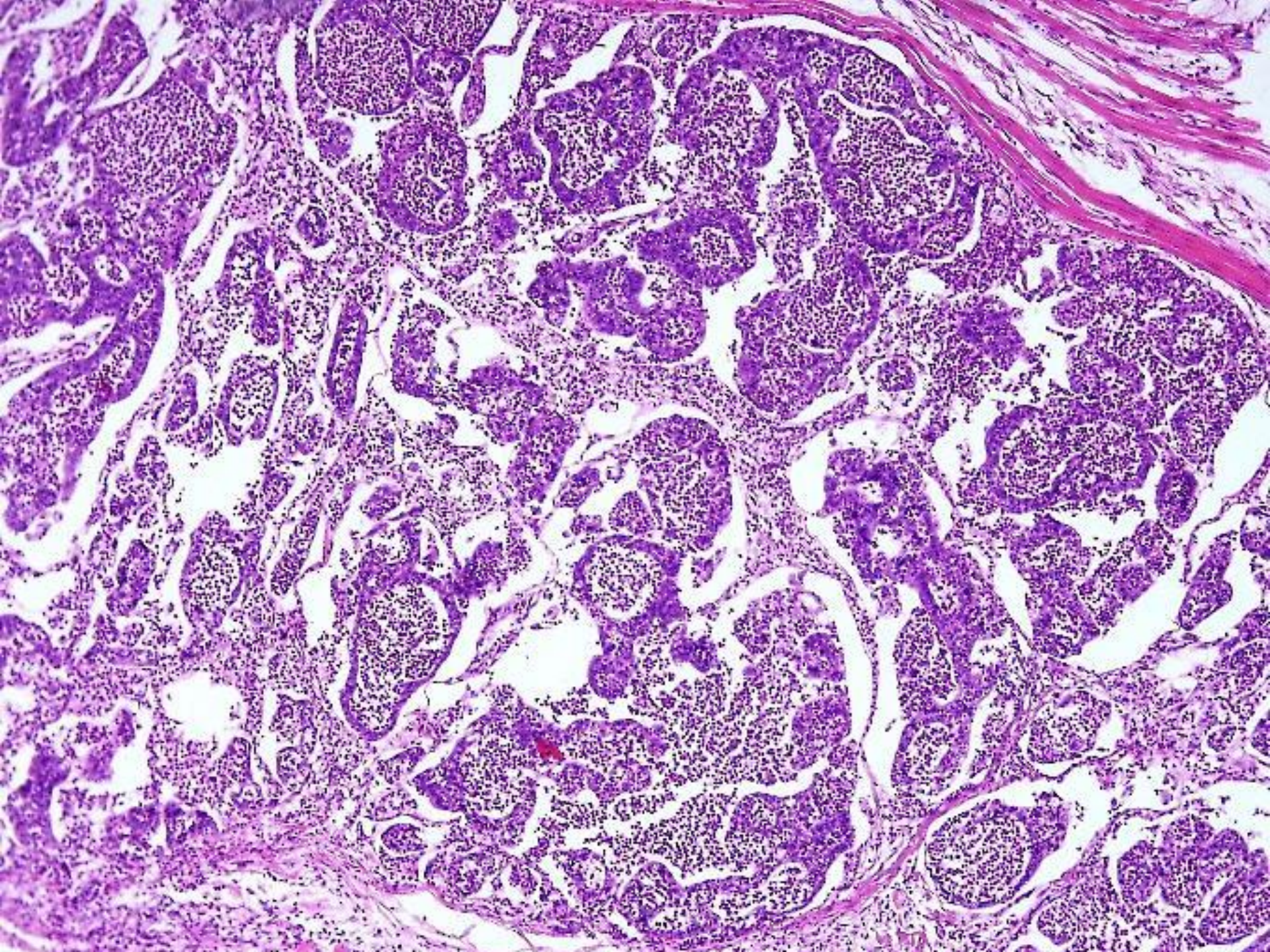
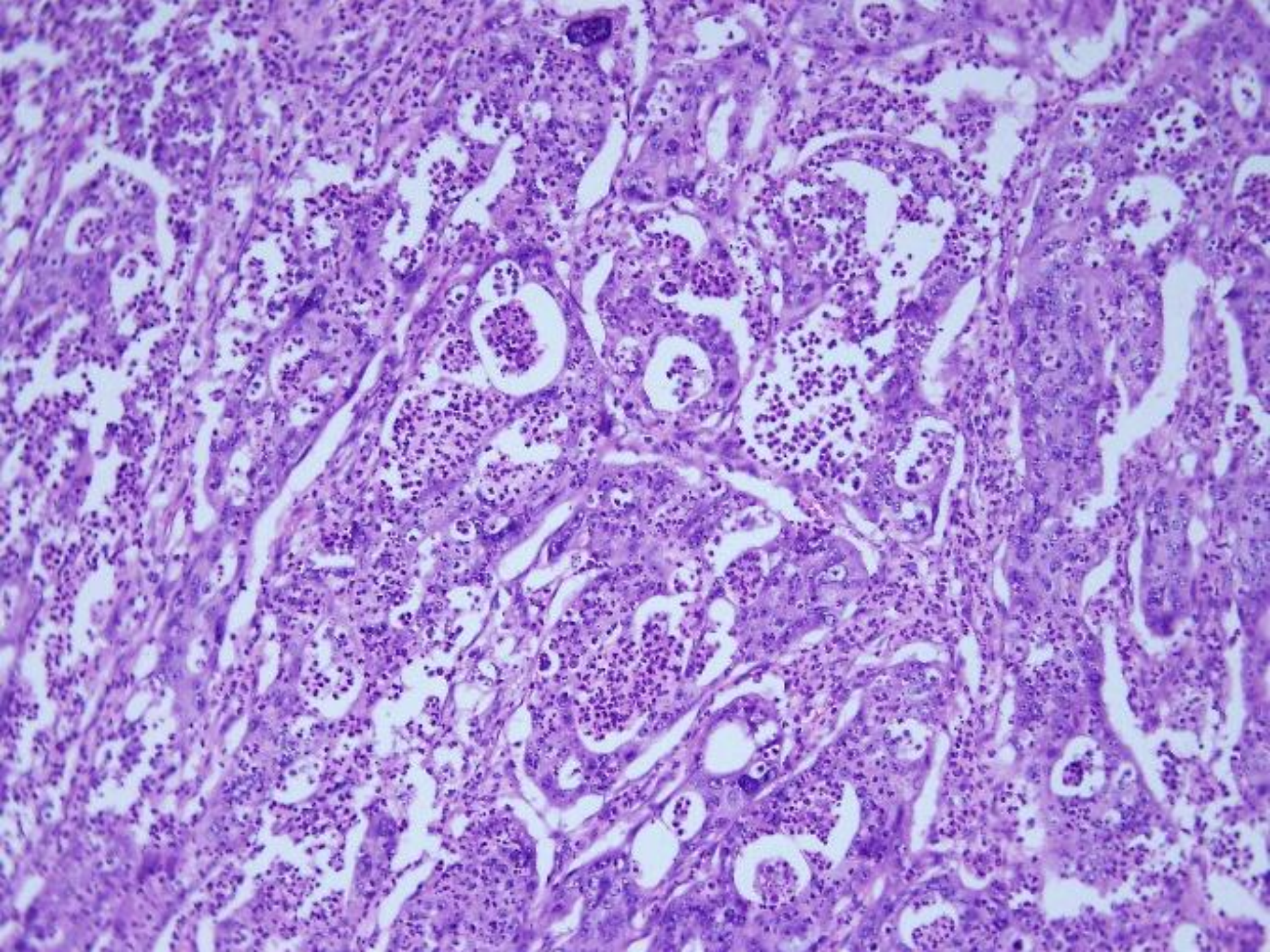


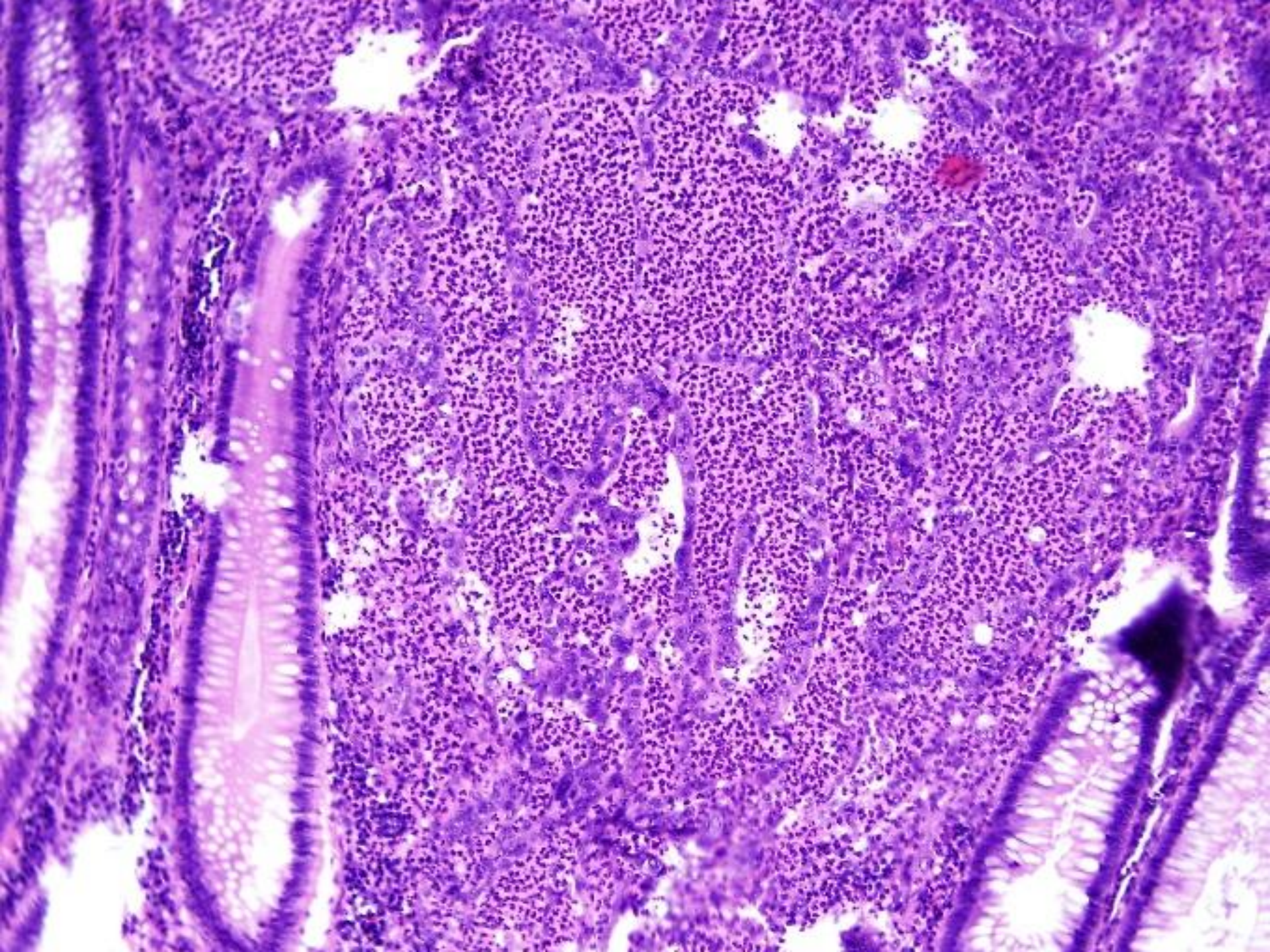


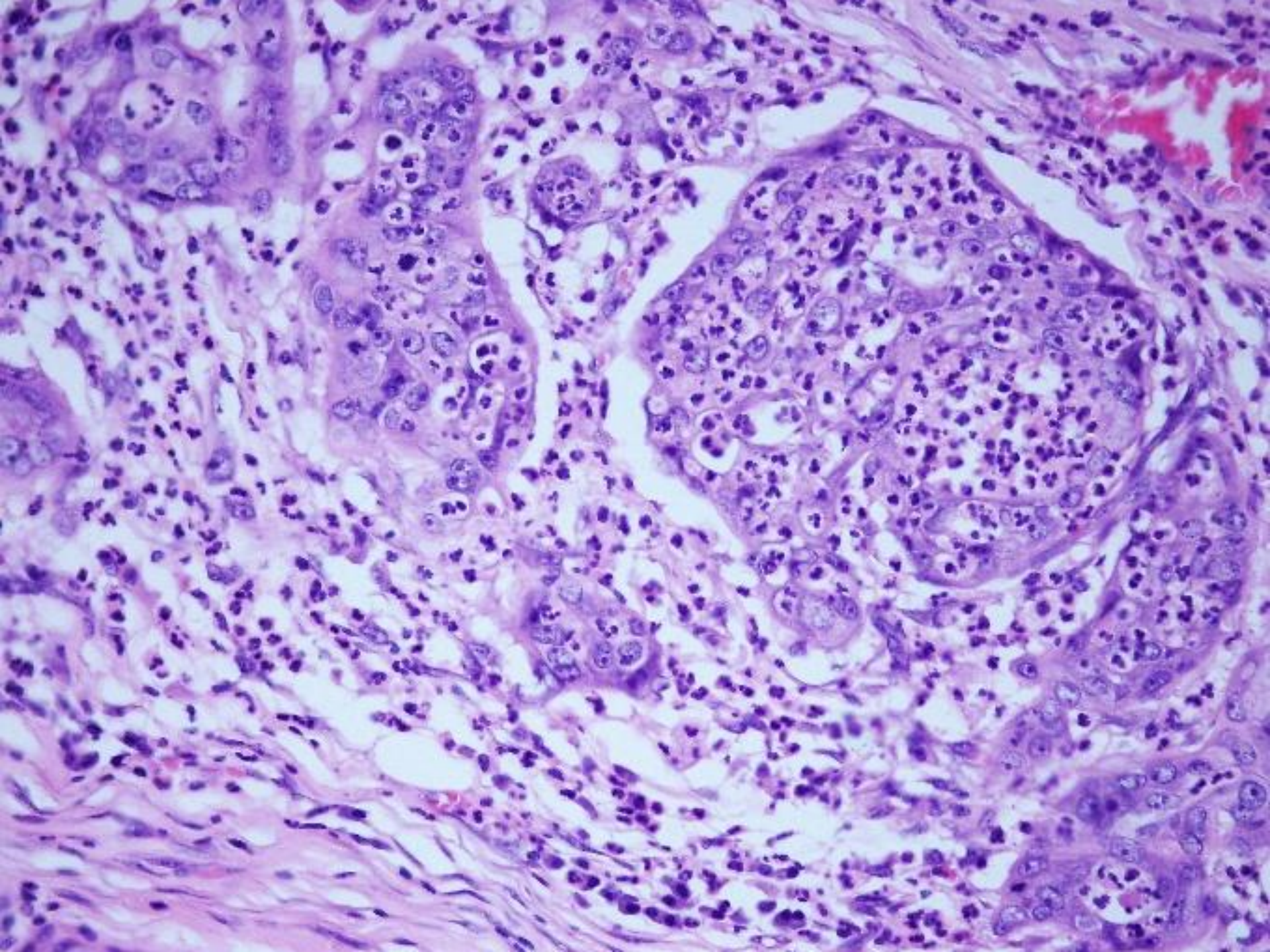


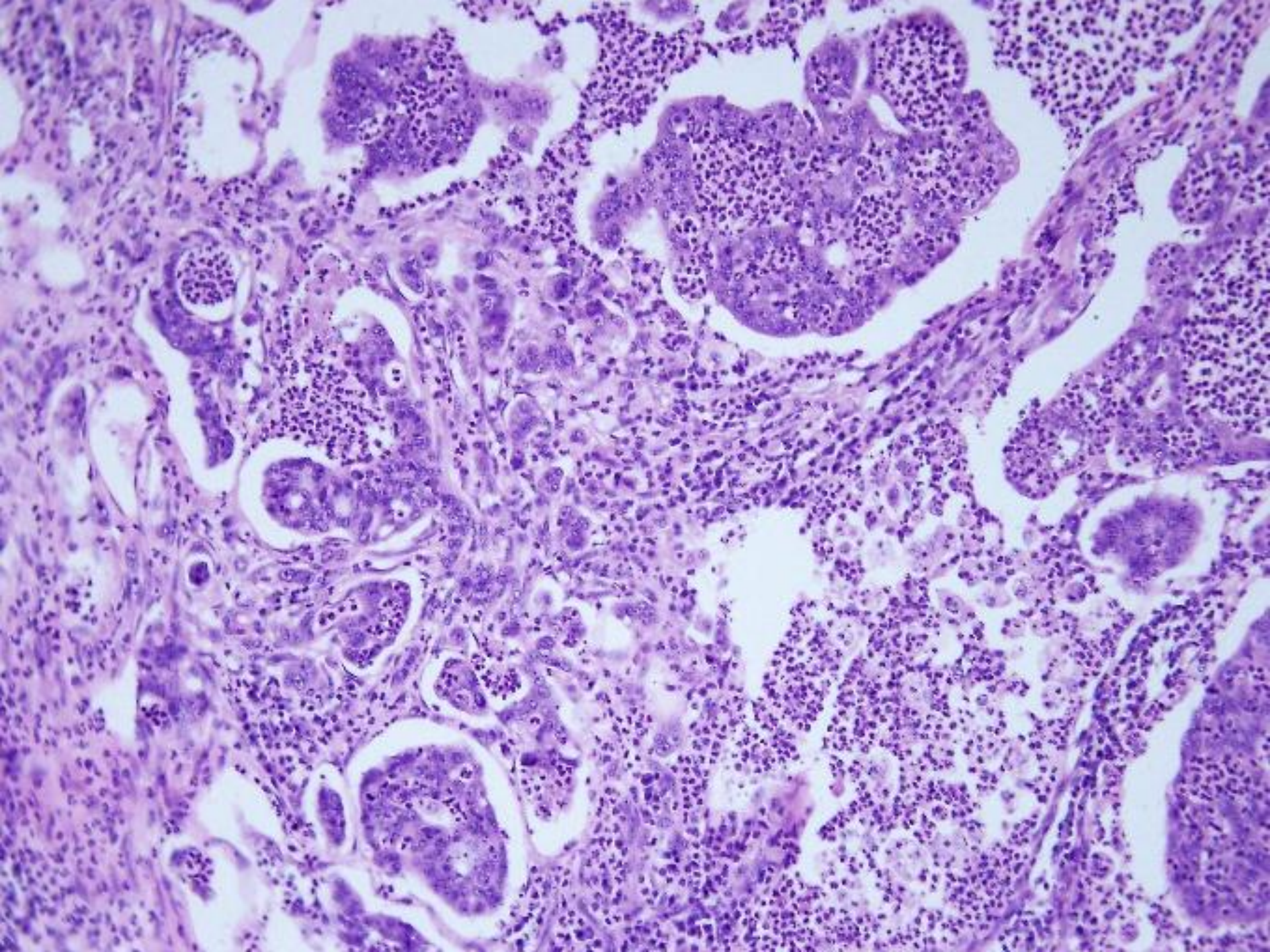


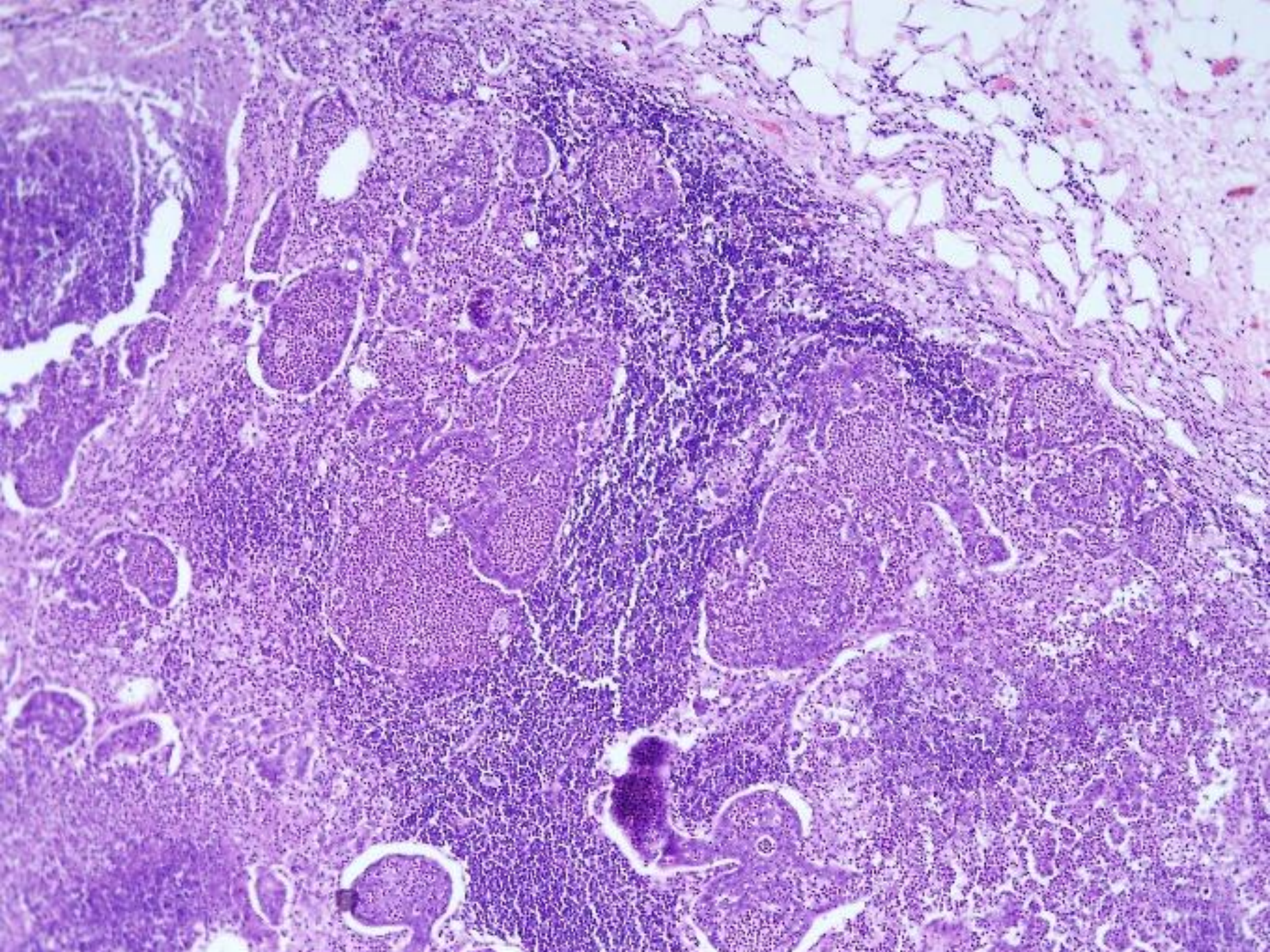


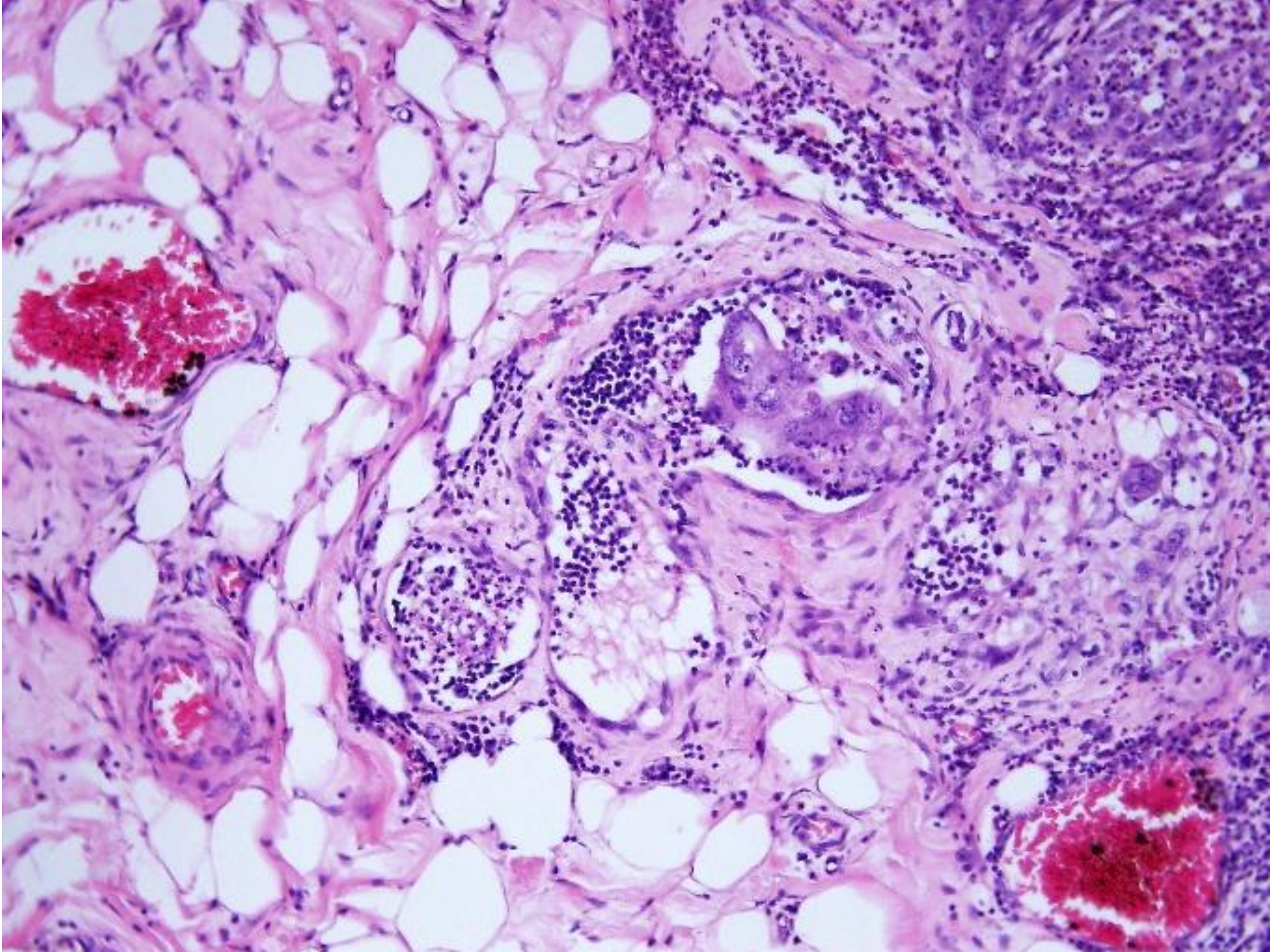


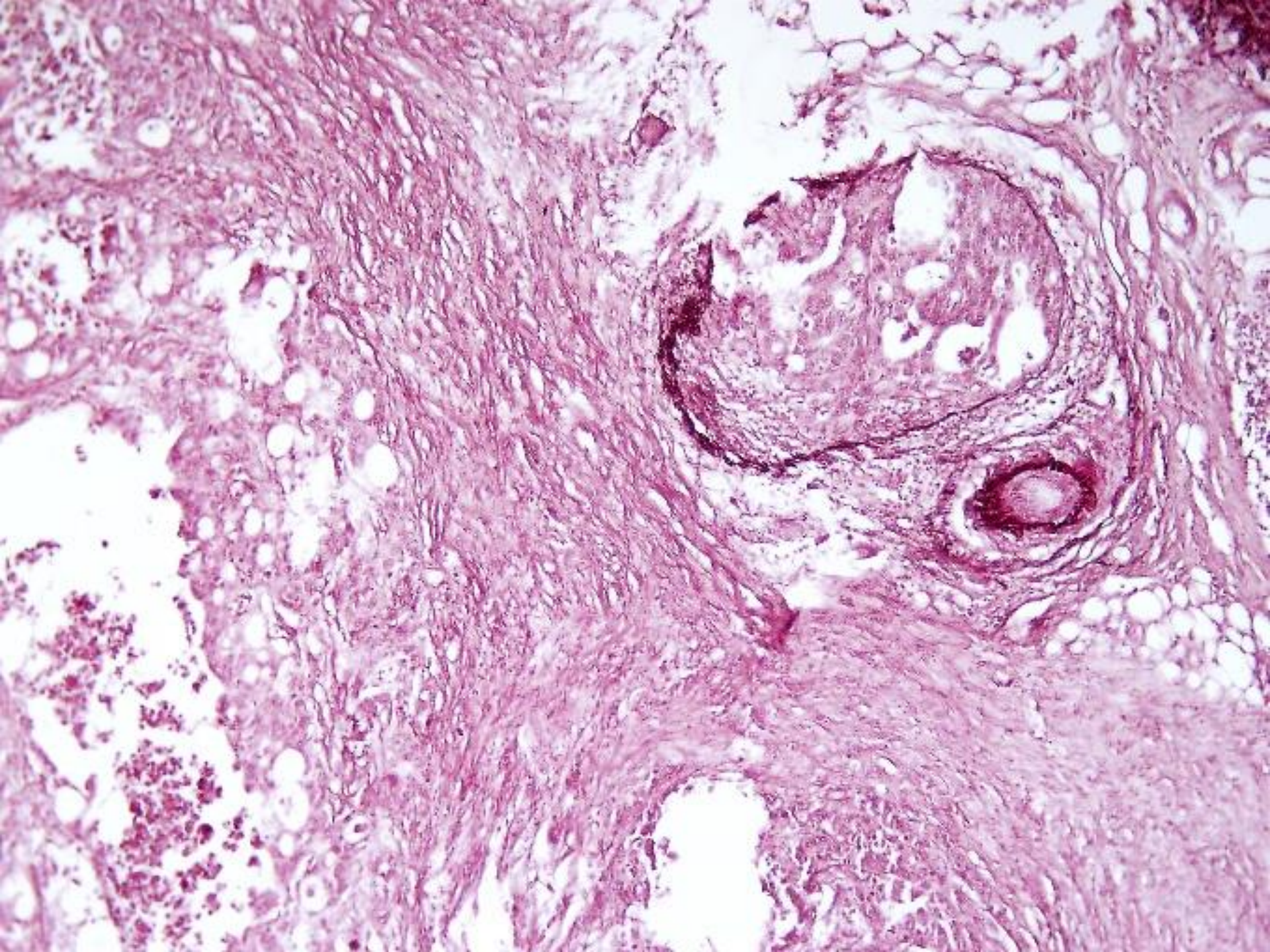












Imunofenotyp

CK7 fokálne +
CK20 +
CDX2 +
CD56 -
MMR : MLH1+, PMS2+, MSH2+, MSH6+

Genetická analýza

Mutácia K-ras p.G13D

Adenokarcinóm s masívnou neutrofilnou reakciou

TNM: pT4a pN2b pM1

Neutrofilné leukocyty a nádor

Priamy aj nepriamy antitumorálny efekt PMN a G-CSF v početných štúdiách za posledných 40 rokov

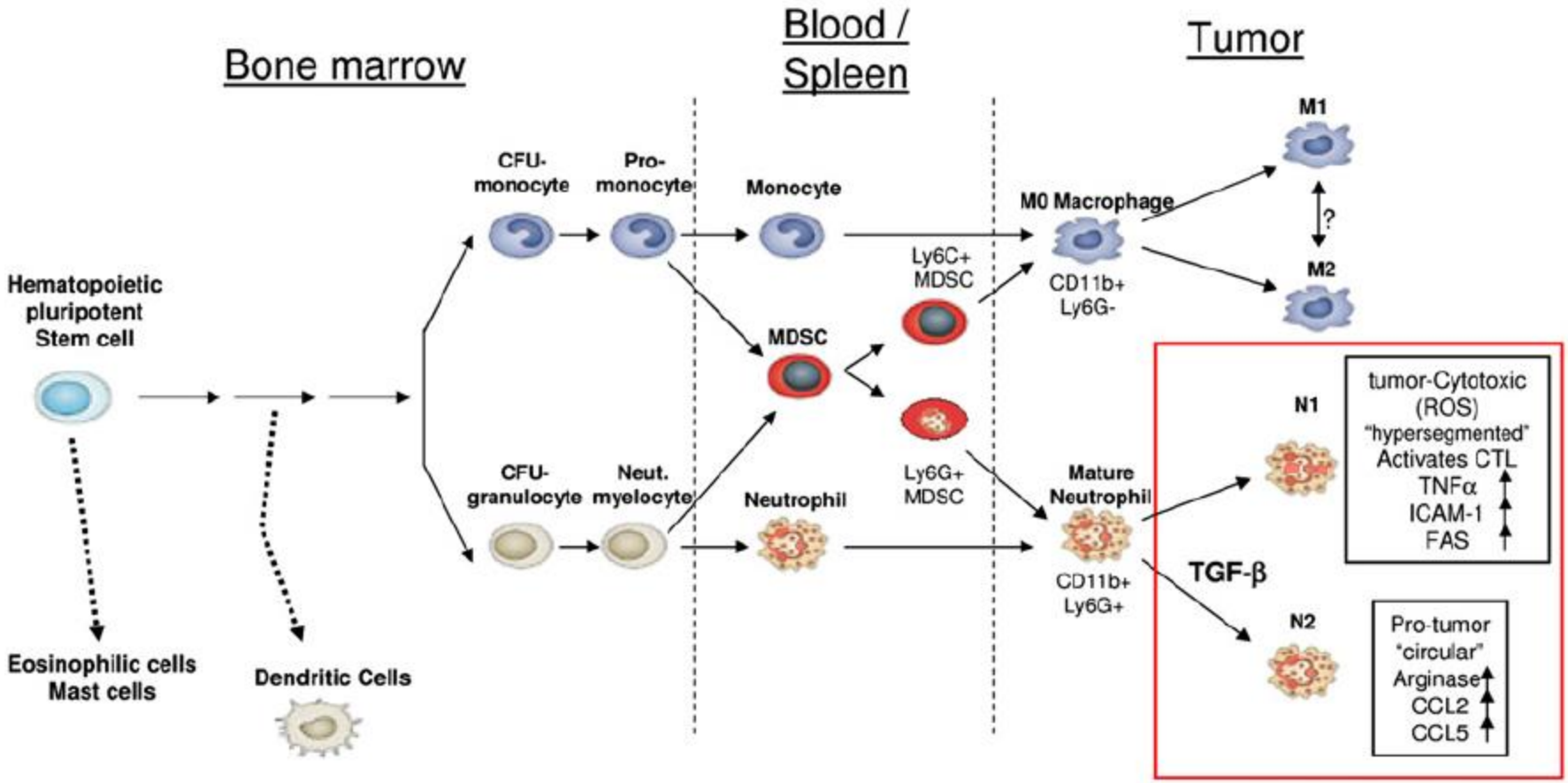
Neutrofília v periférnej krvi: vyššie štádium malignity zlá prognóza (metastatický melanom, bronchioalveolárny Ca, RCC)

Intratumorálne PMN ako negatívny prognostický faktor (lokalizovaný RCC, spinocelulárne Ca hlava/krk, gliomy, karcinómy pankreasu, KRK?)

Intratumorálne PMN ako priaznivý prognostický faktor (žalúdočné Ca?)

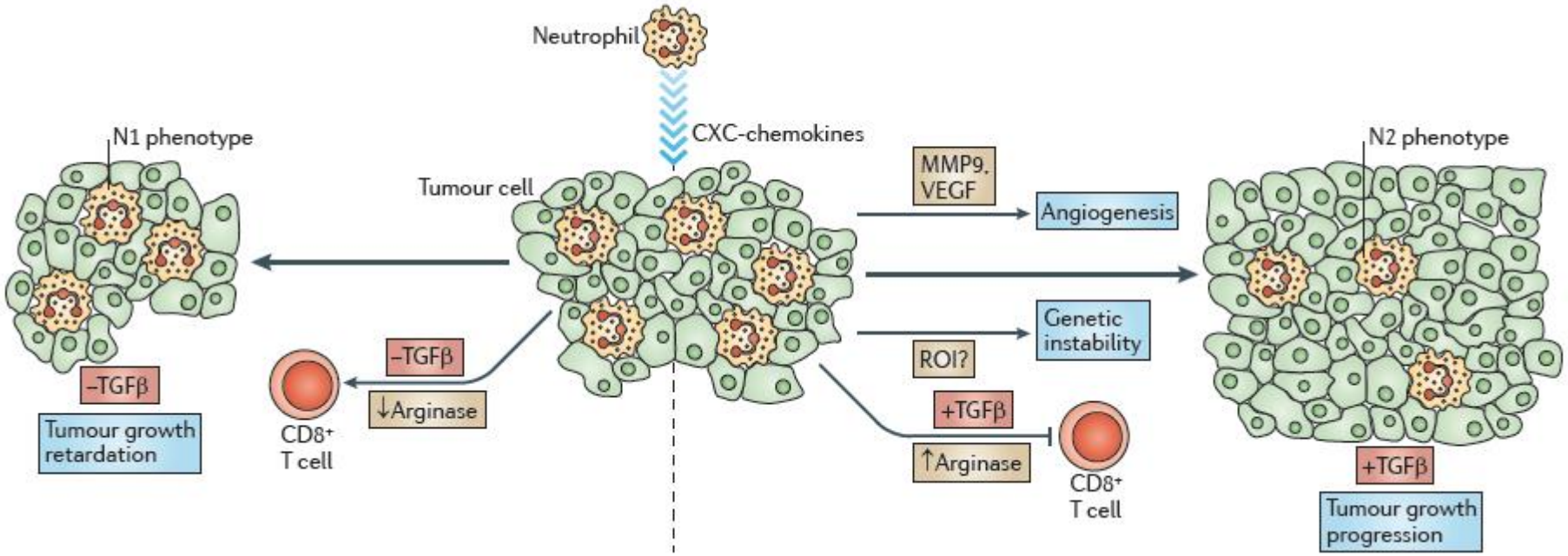
Souto JC Med Res Rev, 2009
Fridlender ZG Carcinogenesis, 2012

Pôvod a diferenciácia myeloidne derivovaných tumor asociovaných buniek



Fridlender ZG et al. Cancer cell 2009

TAN (neutrofilné leukocyty združené s nádorom)



Mantovani A et al. Nat Rev Immunol 2011

Table 1
Antitumoral effects of N1 neutrophils in cancer progression.

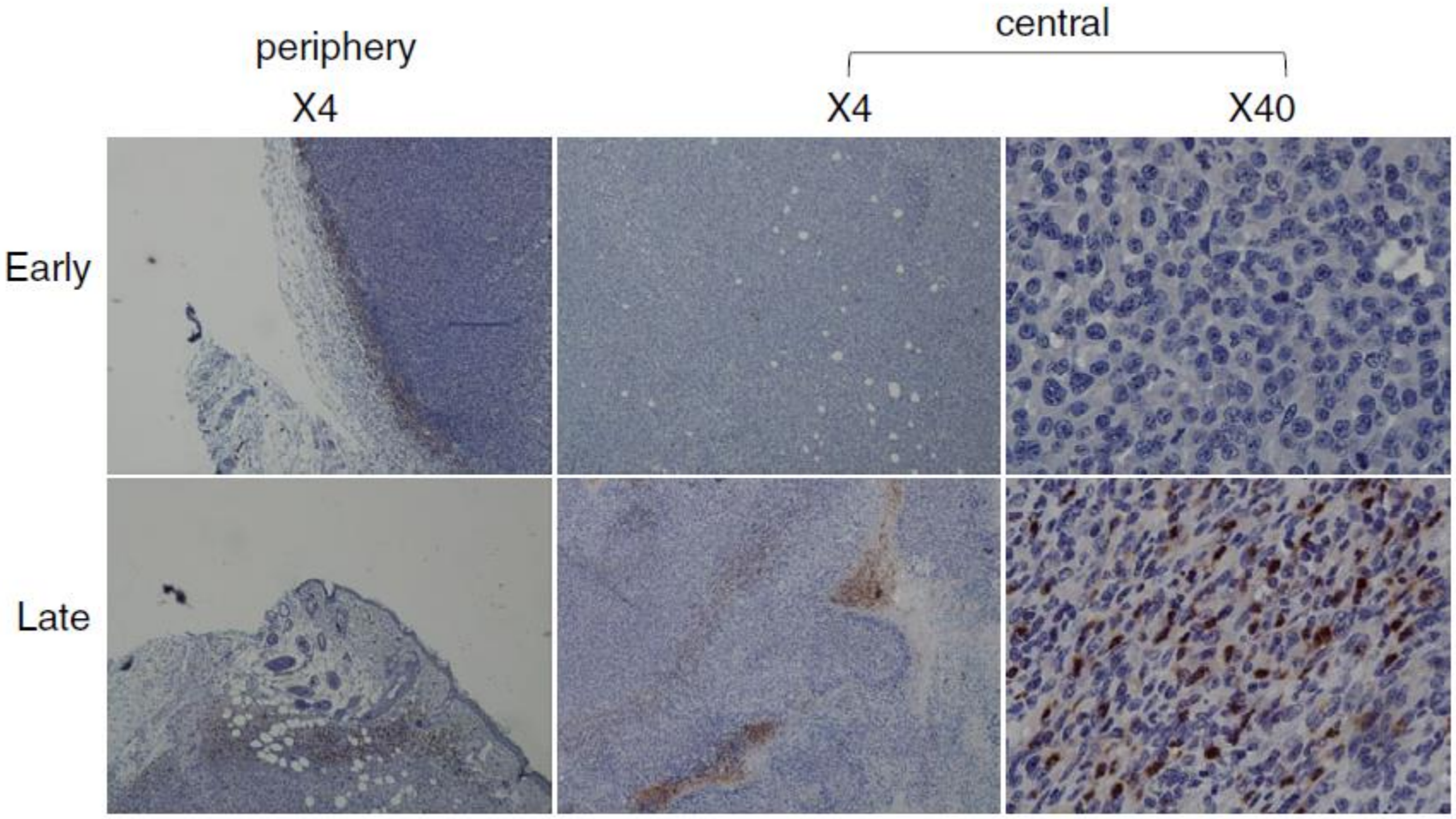
	Mechanism
Tumorigenesis	Neutrophil MMP-8 protects against chemical skin carcinogenesis by preventing chronic neutrophilia
	Neutrophils produce singlet oxygen upon co-culture with tumor cells and diminish tumor cell proliferation
	Neutrophils are antitumoral in the early phase of carcinoma development, resulting in smaller tumor volumes
Cytotoxicity towards tumor(-associated) cells[a]	Isolated neutrophils preferentially kill primary instead of metastatic melanoma cells
	Tumor rejection upon paracrine release of cytokines (e.g. IL-2, TNF-α) is associated with degranulated neutrophils with released granules in close contact with damaged tumor cells
	Tumor cell cytotoxicity upon release of G-CSF is associated with cytoplasmatic projections of activated neutrophils in close contact with dead tumor cells and with cell–cell contacts and fusion of cell membranes between neutrophils and G-CSF-producing cells
	Neutrophil-mediated cytotoxicity of tumors upon cytokine release (e.g. IL-10, IFN-α, IFN-β) targets tumor-associated blood vessels and leukocyte infiltration is mediated through VCAM-1 and E-selectin on endothelial cells
	Cytotoxic capacity of PMNs towards melanoma cells, triggered by knockdown of SPARC
	Neutrophils are cytotoxic and cytostatic towards various tumor cell types
	Neutrophils kill tumor cells, but not normal fibroblasts
	ROS from activated neutrophils causes tumor cell lysis through oxidative damage
	Peripheral blood neutrophils of melanoma-injected mice show an extensive oxidative burst and are cytotoxic for tumor cells
	Neutrophils activated via LFA-1 or Mac-1 release HOCl, resulting in tumor cell lysis, microvessel injury and matrix degradation
	Circulating neutrophils increase after trypan blue treatment of melanoma-bearing mice, have increased cytotoxicity for tumor cells and produce more toxic oxygen radicals, correlated with accelerated clearance of tumor cells that are arrested in lungs
	Neutrophils are activated, more cytotoxic to tumor cells in an oxygen radical-dependent manner, more lobulated and hypersegmented, express more proinflammatory cytokines and chemokines and less arginase in a TGF-β negative milieu
Tumor cell apoptosis[a]	Association of neutrophils with apoptosis in primary prostate tumors
	Binding of antibodies to neutrophil Fc receptors mediates antitumoral cytotoxicity
	Neutrophils mediate antibody-dependent cellular cytolysis of tumor cells via a series of membrane rupture/resealing events ('multihit model')
	Granulocytes are main effectors in treatment of renal carcinoma by bispecific antibodies and G-CSF/GM-CSF
	Neutrophils mediate ADCC of malignant B cells
	PMNs mediate ADCC of melanoma and neuroblastoma tumors through CD11b, CD11c, Fc receptor II and III
	Neutrophils are activated, more cytotoxic to tumor cells and more neutrophils are FAS^+ in a TGF-β negative milieu
Tumor rejection and antitumoral immune memory[a]	Association of granulocyte infiltration with rejection of engineered tumor cells which release cytokines, such as IL-1, IL-4 etc., and immunity against parental tumors
	Association of neutrophil infiltration upon SPARC expression with melanoma tumor rejection
	IL-10-induced rejection of mammary adenocarcinoma by combined action of neutrophils, $CD8^+$ and NK cells and antitumoral memory based on $CD8^+$ cells and neutrophils via tumor-specific antibodies
	Association of neutrophil infiltration with rejection of IL-2-secreting mammary adenocarcinoma, followed by T cell-mediated antitumoral memory with activation of $CD4^+$ cells by granulocytes
	Granulocytes are involved in rejection of secondary tumors
	Antitumoral activity in a TGF-β negative milieu is dependent on activation of $CD8^+$ T cells by neutrophils which express more proinflammatory cytokines and chemokines
Tumor growth	Association of neutrophil influx with impaired survival and tumor growth of IL-8/CXCL8 transduced primary melanoma cells
	Association of neutrophils with decreased *in vivo* tumor growth of IL-8/CXCL8-expressing human ovarian cancer cells
	Association of neutrophils with suppressed subcutaneous tumor growth of huIL-8/CXCL8-, huMIP-1α/CCL3- or muMIP-1α-transfected hamster ovarian tumor cells
	Association of neutrophil increase in murine melanomas or in the circulation with tumor growth inhibition after injection of IL-1β (intratumoral, intradermal, intramuscular)
	Direct transfer of neutrophils into tumor reduced tumor growth by 49%
	Neutrophils are primary effectors in tumor growth suppression of IL-4 expressing melanoma and fibrosarcoma cells
	Neutrophils impair the growth of various cytokine-expressing tumors
	Slower tumor growth by repressed neutrophil-mediated angiogenesis in the presence of constitutive amounts of IFN-β
	Neutrophils are associated with slower tumor growth in a TGF-β negative milieu
Angiogenesis	Slower tumor growth by repressed neutrophil-mediated angiogenesis, associated with inhibited neutrophil expression of VEGF and CXCR4 through downregulation of Stat3 and c-myc, in the presence of constitutive amounts of IFN-β
Anticancer therapy	Lysis of cancer cells upon injection with oncolytic viruses is accompanied by indirect killing of uninfected tumor cells through induction of apoptosis by neutrophils
	Neutrophils are crucial to establish tumor-specific adaptive immunity upon photodynamic therapy

Table 2
Protumoral effects of N2 neutrophils in cancer progression.

	Mechanism
Tumorigenesis	MMP-9 that is predominantly expressed by neutrophils in dysplasias and carcinomas stimulates epithelial carcinogenesis by stimulating hyperproliferation, angiogenesis and tumor progression to more aggressive end-stages
	Neutrophil infiltration upon expression of IL-8/CXCL8 is associated with genetic instability in tumors
Tumor cell apoptosis[a]	Neutrophil infiltration supplies MMP-9 which supports the formation of lung tumors, likely by preventing apoptosis of tumor cells
Tumor growth	Association of neutrophil infiltration with increased tumor growth of highly tumorigenic and metastatic melanoma cells upon increasing levels of IL-8/CXCL8
	Association between influx (and activation) of tumor-associated neutrophils (increased levels of MMP-9) with enhanced tumor growth and increased peritumoral angiogenesis of muGCP-2-expressing melanoma cells
	Association of PMNs and monocytes through CXCR2 with higher tumoral growth rate of murine squamous cell carcinoma with increased expression of murine KC
	Infiltrated neutrophils lead to faster tumor growth with better developed blood vessels in an IFN-β negative milieu
	Neutrophils favor tumor growth in the presence of TGF-β
Angiogenesis	Infiltration of $MMP\text{-}9^+$ leukocytes correlates with the presence of huGCP-2/CXCL6 at sites of neovascularization within human gastrointestinal tumors
	Neutrophil infiltration is associated with strong peritumoral angiogenesis of muGCP-2-expressing melanoma tumors and with increased tumor growth
	Association of PMNs and monocytes through CXCR2 with enhanced angiogenesis in murine squamous cell carcinoma with increased expression of murine KC
	MMP-9 that is predominantly expressed by neutrophils in dysplasias and carcinomas stimulates epithelial carcinogenesis by stimulating e.g. angiogenesis
	Neutrophils within neoplastic lesions switch on angiogenesis during pancreatic islet tumorigenesis, through MMP-9 which likely liberates VEGF
	Neutrophils and their granule contents are angiogenic in developing chicken embryos and TIMP-free active neutrophil MMP-9 induces FGF-2-dependent angiogenesis
	MMP-9-expressing neutrophils are associated with the invasion of new blood vessels into collagen gels, and MMP-9 is required, together with MMP-2, for vascularization *in vivo*
	Infiltrated neutrophils have higher expression of *VEGF*, *MMP-9* and *CXCR4* in an IFN-β negative milieu, and cause better developed blood vessels and faster tumor growth
Invasion	MMP-9 that is predominantly expressed by neutrophils in dysplasias and carcinomas stimulates tumor progression to more aggressive (invasive) end-stages
	MMP-9-expressing neutrophils are associated with angiogenesis in collagen gels, and MMP-9 is required, together with MMP-2, for tumor cell invasion *in vivo*
	Tumor-elicited PMNs, in contrast to "normal" circulating PMNs, stimulate invasion of rat mammary adenocarcinoma cells *in vitro*
	Association between production of (neutrophil) chemokines and malignancy of cancer cells, selective advantage is explained by the countercurrent model
Metastasis	Association of neutrophil infiltration with more metastatic lung foci of highly tumorigenic and metastatic melanoma cells upon increasing levels of IL-8/CXCL8
	Association of neutrophils with shedding of bronchioloalveolar carcinoma cells into the alveolar lumen in patients
	Association of PMNs and monocytes through CXCR2 with more metastasis in murine squamous cell carcinoma with increased expression of murine KC
	Tumor-elicited PMNs, in contrast to "normal" circulating PMNs, stimulate metastasis of rat mammary adenocarcinoma cells
	Metastasis of mammary adenocarcinoma is proportional to PMN levels and associated with PMN secretion of MMP-9 and heparanase
	I.v. co-injection of PMNs raises the number of experimental lung metastasis of mammary adenocarcinoma clones
	Association between production of (neutrophil) chemokines and malignancy of cancer cells, selective advantage is explained by the countercurrent model
	Neutrophils induce detachment of bronchioalvaolar adenocarcinoma cells. Involved neutrophil molecules are e.g. ICAM-1/LFA-1, TNF-α/TNF-α receptor inhibitor, IL-1α/IL-1α receptor, neutrophil elastase
	Stimulated neutrophils induce aggregation of human breast carcinoma cells through neutrophil protease activities
	Neutrophil influx in tumor-bearing lungs is a predominant source of MMP-9 which mediates pulmonary tumor formation, associated with less tumor cell apoptosis
	Neutrophils facilitate the extravasation of melanoma cells, by "two-step adhesion" to endothelial and tumor cells via LFA-1, Sle^X and Mac-1
Anticancer therapy	Intra-abdominal neutrophil influx upon tumor surgery enhances local tumor recurrence
	$CD11b^+GR\text{-}1^+$ cells render tumors refractory to blockade of angiogenesis by anti-VEGF Abs, by mediating an angiogenesis pathway that bypasses VEGF

Piccard H et al. Crit Rev Oncol Hematol 2012

TAN nadobúdajú protumorigénne vlastnosti v priebehu nádorovej progresie



Mishalian I et al. Cancer Immunol Immunother, October 2013

Záver

- TAN zohrávajú významnú úlohu v biológii zhubných nádorov
- Predstavujú zvlášť skupinu neutrofilov, ktoré v mikroprostredí tumoru môžu nadobúdať protumorigénny resp. antitumorigénny fenotyp
- Pochopenie spôsobu a mechanizmov ako tieto bunky podporujú rast nádoru resp. ako proti nemu bojujú môže priniesť nové terapeutické možnosti